

# **Geomicrobiology of the basal ice layer at Svínafellsjökull glacier, SE Iceland**

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## Summary abstract

Glaciers occupy 11% of Earth's total surface and represent a significant but, as yet, poorly characterised ecosystem. As late as the mid-90s glaciers had been regarded as microbiologically sterile environments but there has since been major progress characterising diversity and functioning of glacier microbiota. The supraglacial environment has to-date been prioritised, but crucially the subglacial microbiota remains generally unknown despite their central importance in geochemical cycling. The dark and oligotrophic conditions typical to subglacial environments in general, and sediment entrained basal ice, in particular, are likely to select for chemolithotrophic carbon fixers that, by definition, will enable diverse heterotrophic microbial community development. Therefore, the basal ice microbiota, are likely to play fundamental roles in mineral weathering and geochemical cycling not only within basal ice but also subsequent foreland soil formation upon release. The main aim of this thesis is the first integrated geo-microbiological characterization, of geomorphologically distinct basal ice facies targeting an Icelandic temperate glacier, Svínafellsjökull. Here we show, via novel culture-dependent and -independent next generation molecular rRNA gene marker (16S and ITS) phylogenetics, that basal ice facies harbour a rich and diverse community of bacteria (Proteobacteria and Acidobacteria) and fungi (Ascomycota and Basidiomycota). An abundance of chemolithotrophic species (*Thiobacillus*, *Gallionella*, *Nitrosospira*) characterise the basal ice microbiome that is directly supported in the identified geochemical status of basal ice. The presence of reduced nitrogen species in the ice matrix and iron- and sulphur-rich minerals in basal ice sediment provides added functional support of the predominance of chemolithotrophs in basal ice. Based on total basal ice cell enumeration and export estimates ( $\sim 10^{16}$  cells  $\text{yr}^{-1}$ ) it is clear that chemolithotrophic and heterotrophic microbial communities identified in foreland soils originate from basal ice, as opposed to supraglacial sources. Modelling highlights the importance of microbial activities on the geo-chemistry of the basal ice, e.g. oxidising minerals, acidifying the environment, and increasing the carbon content within the sediment. Once released, basal ice-derived microorganisms can survive the psychrophilic to mesophilic temperatures of the foreland and identified isolates from basal ice and

the foreland were affiliated with species that play important roles in weathering and soil formation highlighting the functional importance of the basal ice microbiota both, in glacial and periglacial systems.

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## 1. Literature review

### 1.1 The cryosphere habitat

The cryosphere comprises a significant proportion of the Earth where the mean temperature is below 5°C (Anesio and Laybourn-Parry, 2012). Excluding the ocean floor, the current estimated volume of >33 million km<sup>3</sup> includes the ice sheets of Antarctica and Greenland, sea ice in the Arctic and Antarctic Oceans, and alpine glaciers at high altitudes (Boetius et al., 2015). Due to prevailing harsh conditions, the bulk of active biomass in the cryosphere is represented by microorganisms belonging to bacterial and archaeal domains of life (Boetius et al., 2015). The microorganisms inhabiting diverse cryosphere environments are, by definition, adapted to cold temperatures, being either psychrotolerant, or psychrophiles (Anesio and Laybourn-Parry, 2012). The total number of microbial cells inhabiting the cryosphere has been estimated to be approximately 10<sup>25</sup>-10<sup>28</sup> cells (Boetius et al., 2015). The most abundant and diverse bacterial phyla across the cryosphere are Proteobacteria, followed by Actinobacteria in the case of permafrost, Alphaproteobacteria in seawater, Flavobacteria and Gammaproteobacteria in sea-ice communities, and Betaproteobacteria in snow, glacial ice and subglacial ecosystems (Boetius et al., 2015). Sequences affiliated with Euryarchaeota (belonging to Archaea) have been found in Antarctica (Cavicchioli et al., 2000; Yang et al., 2007).

### 1.2 Glacial environments

Glaciers occupy >10% of the total land surface (15 million km<sup>2</sup>) but store around 70% of all freshwater on Earth (Anesio and Laybourn-Parry, 2012; Lutz et al., 2015). Precipitation, mostly in the form of snow and/or water, accumulates as ice in the accumulation zone of a glacier whilst in the ablation area, ice is lost by processes of melting, sublimation, and/or calving (Fig 1.1) (Anesio and Laybourn-Parry, 2012; Boetius et al., 2015). Most of the glacier ice forms by the process of firnification, in which successive episodes of snow deposition compress the underlying snow, which squeezes out the air to generate solid ice. A comparatively minor proportion of the glacier is represented by basal ice, which originates in the lowermost ice strata that is in contact with the bedrock and/or soil substrate (Knight, 1997; Hubbard et al., 2009).

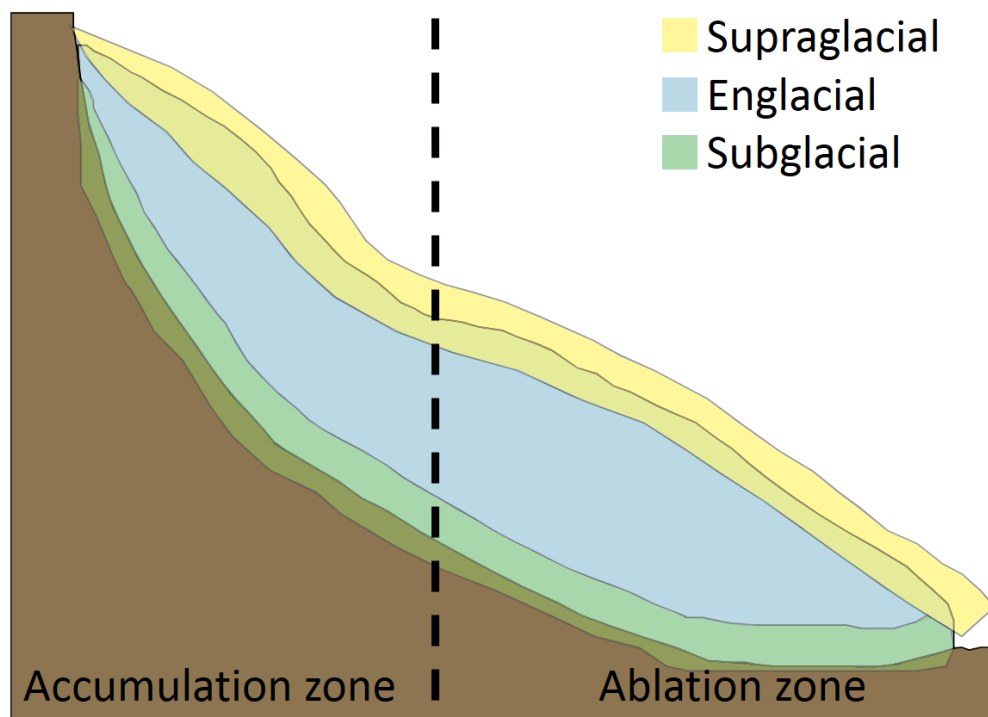
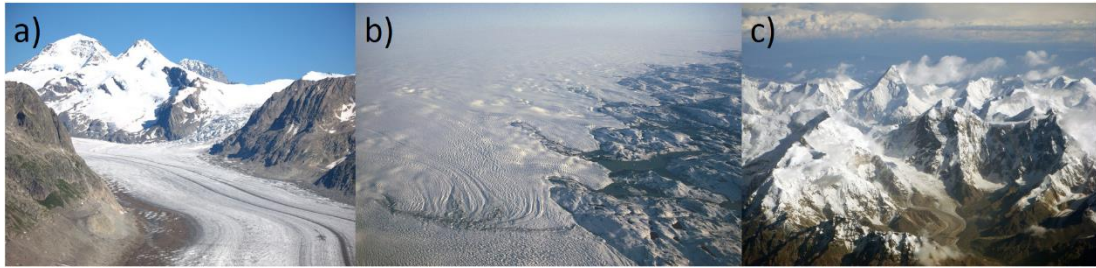


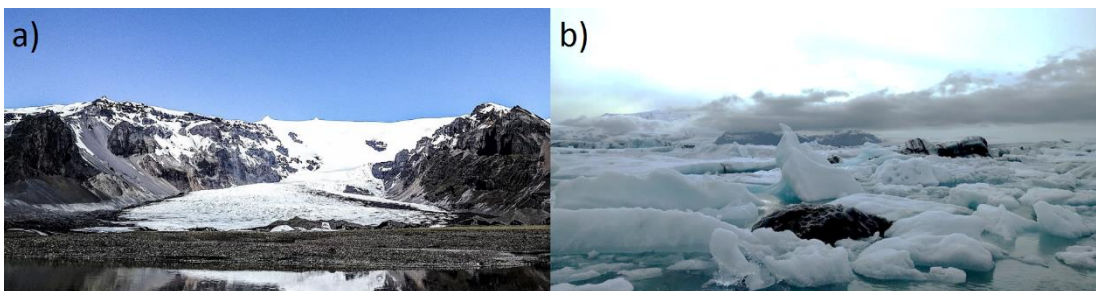
Figure 1.1. Schematic representation of the different environments found in an idealised valley glacier.

### 1.2.1 Glacier diversity and classification

Depending on ice temperature, glaciers can be classified as temperate, where temperatures are maintained close to the melting point and where subglacial meltwater is very likely to be present; cold-based, where liquid meltwater is absent and the glacier is frozen to its bed; or polythermal, where a mixture of thermal regimes exists, typically comprising temperate ice up-glacier, and cold-based ice around the margins. Depending on topography, glaciers can be categorised as valley glaciers, constrained by rock walls; ice sheets, which overwhelm the landscape morphology; or cirques, a bowl-like structure with high rock walls enclosing the glacier (Fig 1.2). Glaciers also vary in their dynamic context as they can terminate on land (terrestrial) or in water bodies, where they release icebergs (calving) (Fig 1.3) (Bennett and Glasser, 2006).



**Figure 1.2. Glacier types based on their topography.** A) Valley glacier in the Alps (Picture by Andrew Bossi - Own work, CC BY-SA 2.5, <https://commons.wikimedia.org/w/index.php?curid=2989117>), B) Ice sheet in Greenland (Picture by Hannes Grobe 20:10, 16 December 2007 (UTC) - Own work, CC BY-SA 2.5, <https://commons.wikimedia.org/w/index.php?curid=3237742>), and C) Cirque glacier in the Tian Shan mountains (picture by Chen Zhao - originally posted to Flickr as 天山山脉西段航拍 / West Tian Shan mountains, CC BY 2.0, <https://commons.wikimedia.org/w/index.php?curid=8723906>)



**Figure 1.3. Different glacier termini.** a) Land-terminating glacier (Kvíarjökull, SE Iceland) and b) Calving glacier terminating in a Lagoon (Breiðamerkurjökull in Jökulsárlón, SE Iceland). Pictures by Mario Toubes-Rodrigo

Within an individual glacier, the edaphic conditions (e.g. nutrient concentration and bioavailability, presence of light, oxygen concentration, hydrostatic pressure) supporting life may vary significantly (Hodson et al., 2008; Anesio and Laybourn-Parry, 2012), and three major zones are typically described in an idealised glacier (Figure 1.1):

**Supraglacial:** The uppermost layer of glaciers (Fig. 1.1), up to 1 m, is exposed to solar radiation and the input of debris and microorganisms transported by wind, rockfalls, and precipitation (Anesio and Laybourn-Parry, 2012; Temkiv et al., 2012; Boetius et al., 2015; Rime et al., 2016). One of the most limiting factors for life is the presence of liquid water which is typically abundant at the glacier surface, particularly in the ablation zone (Fig 1.1). Life has been documented in the uppermost part of glaciers, in particular in cryoconite holes, which are vertical hollows in the ice surface filled with water containing debris at their base (Takeuchi et al., 2001). These are formed where there is an accumulation of dark sediment or soot particulates, capable of absorbing solar radiation (Takeuchi et al., 2001). The presence of dark particles

results in increased localised surface temperatures, reaching  $\sim 2^{\circ}\text{C}$  in the presence of pigment-bearing microorganisms, which leads to thawing of the surface ice (Lutz et al., 2014). As the process of localised thawing progresses, the debris sinks deeper into the ice as the cryoconite hole penetrates the surface ice through freeze-thaw cycles (Takeuchi et al., 2001). The presence of liquid water creates favourable conditions for the establishment of a high diversity and abundance of microorganisms in cryoconite holes (Hodson et al., 2008; Edwards et al., 2011; Edwards, Rassner, et al., 2013; Hamilton et al., 2013; Kaczmarek et al., 2016). The significant levels of microbial activity is comparable to soils and sediments from temperate latitudes (Anesio and Laybourn-Parry, 2012). Microorganisms have also been reported in snow and supraglacial ice away from areas that feature cryoconite holes (Lutz et al., 2014, 2015; Stibal et al., 2015). The deposition of particulate mineral and organic matter and associated microbes via long-distance aeolian transport and precipitation can both respectively fertilize and inoculate the glacier surface with nutrients and microbes (Anesio and Laybourn-Parry, 2012; Lutz et al., 2014). Significant levels of variability have been recorded in the number of microbial cells inhabiting the supraglacial environment, which varies from  $10^4$  cells  $\text{ml}^{-1}$  of ice in clean surface ice, to  $10^8$  cells  $\text{ml}^{-1}$  in cryoconite holes (Boetius et al., 2015). Studies during the melt season showed that the cell content in the supraglacial environment increases especially where it is associated with the accumulation of photosynthetic organisms, which, due to the presence of microbial pigments, colour the snow, such as chlorophylls conferring green/yellow colours; and carotenoids, which will confer pink/red colours (Lutz et al., 2014; Boetius et al., 2015). The capacity of snow to reflect light (albedo) can be reduced (by up to 40%) by the presence of pigmented microorganisms (Lutz et al., 2014). Some of the microbial taxa that have been identified in the supraglacial ice and snow include the *Chlamydomonadaceae* family (Jones, 2001) and *Zygnematophyceae* (Takeuchi, 2013; Lutz et al., 2014). Cryoconite holes, on the other hand, are dominated by photosynthetic *Cyanobacteria* that are replaced by algae in snow (Anesio and Laybourn-Parry, 2012). Predominating cyanobacteria inhabiting cryoconite holes belong to the genera *Oscillatoria*, *Leptolyngbya*, *Phormidium* and *Nostoc* (Boetius et al., 2015). Photosynthetic cyanobacterial colonization leads to the accumulation of significant amounts of C in the supraglacial environment, which,

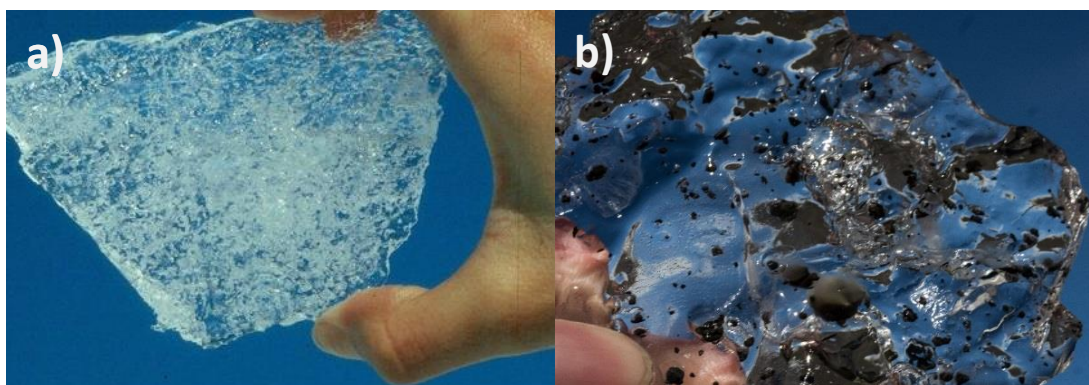
through subsequent melting and runoff processes, becomes available in downstream environments, such as forelands, coastal water, and subglacial environments (Anesio and Laybourn-Parry, 2012; Boetius et al., 2015). All major phyla of heterotrophic bacteria and fungi have been found in cryoconite holes, being dominated by Proteobacteria and Actinobacteria, but with presence of Bacteroidetes, Acidobacteria, Chloroflexi, and Planctomycetes. Isolates from cryoconite holes are affiliated with genera such as *Pseudomonas*, *Polaromonas*, *Micrococcus*, *Cryobacterium* and *Flavobacterium* (Boetius et al., 2015; Kaczmarek et al., 2016). Fungi were identified to Basidiomycete yeast or filamentous Ascomycota (*Helotiales/Pleosporales*) (Edwards et al., 2013). Several fungal genera have been found inhabiting cryoconite holes in both the Arctic and Antarctic, including *Alternaria*, *Aspergillus*, *Rhodotula* (Kaczmarek et al., 2016).

**Englacial:** defines the central part of glaciers, beneath the sunlit zone (Fig 1.1). Most ice in the glacier has undergone a process called firnification and is characterised by low debris content (Hubbard et al., 2009). During firnification, snow of meteoric origin (i.e. precipitation-derived) is buried and compressed by the deposition of subsequent layers of new snow. Therefore, there is growth of ice crystals, as well as a partial expulsion of trapped air. This, and the low debris content confers a blue colouration to the ice under well illuminated conditions (Fig 1.4.a) (Hambrey et al., 1999; Benn and Evans, 2010). The firnification process limits space for microbial growth due to the low availability of liquid water, which, tied to nutrient limitation, leads to extremely low cell abundance with reported cell numbers between  $10^1 - 10^3$  cells  $\text{ml}^{-1}$  ice (Boetius et al., 2015).

**Subglacial:** comprises the lowermost part of the glacier, directly in contact with the substrate which may include bedrock, unconsolidated sediment, or a combination of the two (Fig 1.1). Basal ice, found within in the lowermost part of glaciers, typically has physical, chemical and biological properties that are conditioned by contact with the glacier substrate, and hence is significantly different from the overlying firnified englacial ice (often termed 'glacier ice') (Hubbard and Sharp, 1989; Knight, 1997; Montross et al., 2014). Basal ice has been reported to be rich in sediment, poor in bubble content, with small crystal sizes, and enriched in some solutes, namely  $\text{Ca}^{++}$



and  $Mg^{++}$  (Fig 1.4) (Hallet et al., 1978; Hubbard and Sharp, 1989; Knight, 1997). The high debris content within basal ice results from entrainment of particles from the substrate through a variety of melting and (re-)freezing processes at the glacier bed and terminus (see section 1.3) (Knight, 1997). Moreover, the presence of particles in the ice matrix inhibits the growth of ice crystals, thereby promoting small crystal sizes. Glacier flow over the substrate deforms and shears the ice, further reducing crystal size (Knight, 1997). The low air content is related to processes of basal melting and re-freezing whereby gas is squeezed out under pressure (Fig 1.4) (Hubbard, 1991). Elevated concentrations of some solutes are inherited from bedrock weathering and the weathering of entrained sediment particles (Hallet et al., 1978; Rea et al., 2004). The unique characteristics of basal ice mean that it represents a potentially important biome for micro-organisms (Hodson et al., 2008), yet despite recent growing interest in basal ice microbiology (Lee et al., 2012; Koh et al., 2012; Doyle et al., 2013; Montross et al., 2014), little is known about the nature and significance of subglacial microbial life. Thus, the geomicrobiology of basal ice forms a primary focus of this thesis.



**Figure 1.4. Comparison between atmospherically-derived englacial ice and subglacially-derived basal ice.** A) Bubble-rich, debris poor englacial ice (Source [www.peterknight.com](http://www.peterknight.com)). B) Bubble-poor, debris-rich dispersed facies basal ice (photography taken by Dr David Elliott).

### 1.3 Basal ice and its formation

Basal ice is defined as “ice that has acquired a distinctive suite of physical and/or chemical characteristics as a result of processes operating at or near to the bed of an ice mass” (Hubbard et al., 2009). Basal ice is highly heterogeneous and can vary significantly in nature within an individual glacier and between glaciers. To describe this variability, glaciologists have adapted sedimentary geology nomenclature with

regard to basal ice 'cryofacies' defined as "the smallest unit of ice that is characterized by spatially-prevalent or –repeated internal homogeneity, either in terms of characteristic layering or a uniformly massive structure" (Hubbard et al., 2009).

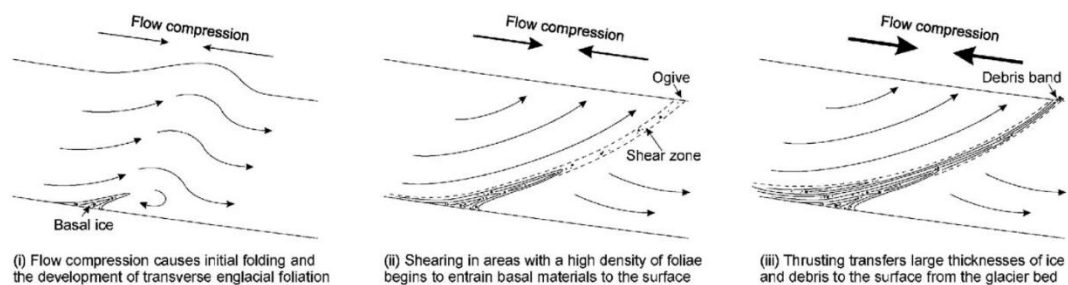
The variability in basal ice characteristics reflects both the diverse geological sources of sediment that becomes entrained in the ice which, in turn, affects the chemistry and biology of the ice (Montross et al., 2014) but also the multiplicity of processes that generate basal ice. The following section describes these mechanisms and reviews the controls on their operation.

**Strain-induced metamorphism:** Glacier flow by internal deformation (Glen, 1955; Nye, 1957) causes local melting of the ice and expels the air from the liquid, thereby decreasing the bubble-content. Furthermore, water can be driven through the inter-crystalline vein network along a pressure gradient from the bed up into the body of the glacier (Robin, 1976; Knight and Knight, 1994). Hence, solute-rich water bearing fine debris from the subglacial region can also move upwards to contribute to debris-bearing basal ice formation (Hubbard et al., 2000). Specifically, this process has been suggested to form the so-called 'clear facies' basal ice (also known in some other studies as 'dispersed facies') (Fig 1.4b) (Knight and Knight, 1994; Hubbard et al., 2000; Cook et al., 2011b).

**Tectonic incorporation of debris into ice:** The entrainment and transfer of sediment through glaciotectonic processes (i.e. folding and faulting) can take place under a number of different scenarios (Fig 1.5). In polythermal glaciers, it has been suggested that thrust-faulting may occur associated with the presence of two thermally distinct regions close to the glacier terminus. Here, warm-based, sliding ice from up-glacier flows against cold-based ice frozen to the bed around the glacier margin (Moore et al., 2011). Under these circumstances, it has been suggested that the differential motion across this thermal boundary induces stress sufficient to shear the ice and entrain sediment (Hambrey et al., 1996, 1999). This hypothesis has been used to explain the presence of dark sediment-rich bands in polythermal glaciers (Hambrey et al., 1999). Layers of sediment or sediment-bearing basal ice in the subglacial region can experience longitudinal compression, leading to the formation of folds. These structures represent planes of weakness that are susceptible to thrust faulting as

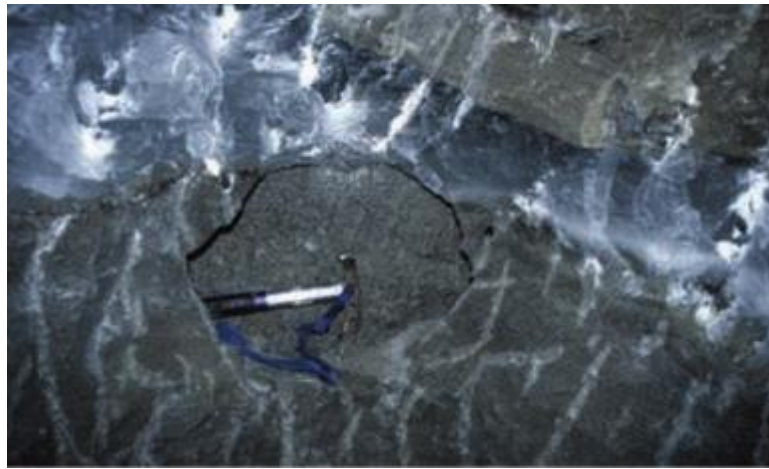
deformation continues. Thrusting and folding may entrain sediment into the glacier and transfer it towards the surface (Swinzow, 1962; Hambrey and Huddart, 1995; Hambrey et al., 1996, 1999). These processes can be particularly important when the glacier flows against an adverse bedslope close to the terminus (Moore et al., 2010), and when additional conditions are met, such as an optimum orientation of pre-existing fractures in hydraulic communication with the high-pressure subglacial water system (Moore et al., 2011). The only situation where thrust faulting has been unequivocally observed was during a surge of Variegated Glacier, Alaska (Raymond et al., 1987).

Glaciotectonic entrainment processes may also be important downstream of an icefall (Goodsell et al., 2002; Cook et al., 2011b) and have been proposed to be a reason for the appearance of a banded pattern of dark and clear ogives on the surface of some glaciers. Ice accelerates and extends as it flows down an icefall, but experiences enhanced compression at the base of the icefall, which may lead to entrainment of sediment into the ice by folding and thrusting. At the surface, this may be reflected as a pattern of alternating dark and clear ogives. Dark ogives have been described to have a low bubble content, small ice crystals, and higher sediment content, suggesting a subglacial origin (Goodsell et al., 2002). In some cases, when a glacier terminates in a subglacial basin or overdeepening, further sediment entrainment and elevation may occur associated with reactivation of the ogive shear planes through enhanced longitudinal compression (Swift et al., 2006; Cook et al., 2011b) (Fig 1.5).



**Figure 1.5. Stages of debris band development under increasing longitudinal flow compression:** (i) initial folding and the development of transverse englacial foliation at the base of the icefall; (ii) entrainment of basal materials by shearing along englacial foliae; and (iii) transfer of basal materials to the glacier surface by thrusting. Source Swift et al. (2006).

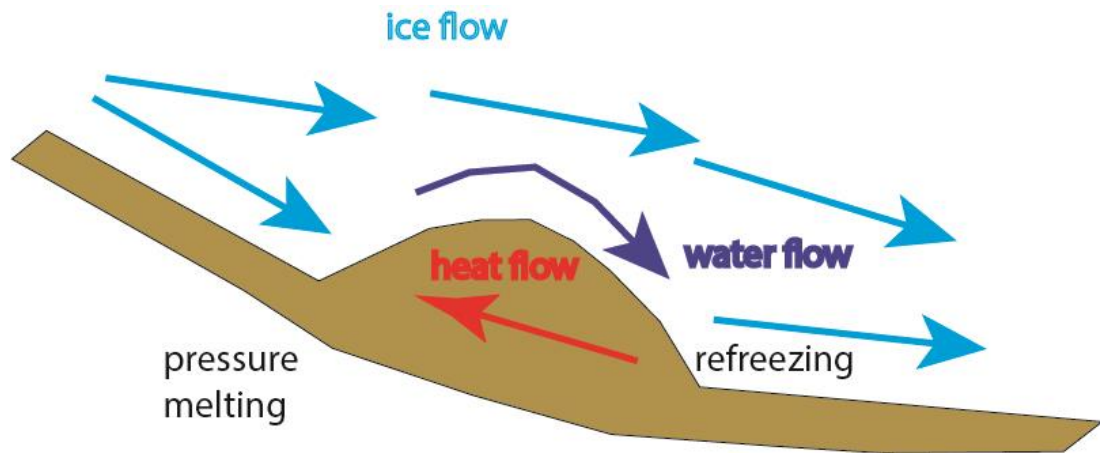
**Apron entrainment:** advancing glaciers can entrain ice detached from the glacier front (Shaw, 1977) as well as ice from marginal and submarginal permafrost (Waller et al., 2012) or frozen sediment (Hambrey and Fitzsimons, 2010). If detached ice is buried in debris, it will be protected from melting/sublimation processes and when re-entrained in the glacier by overriding remains as an icy core within a sediment block. This process is particularly relevant in dry and cold climates, such as the Canadian High Arctic or Antarctica (Shaw, 1977; Evans, 1989).



**Figure 1.6. Frozen sediment entrained by apron entrainment in the Dry Valleys, Antarctica.** A sandy core can be observed surrounded by a matrix of bubble poor basal ice. Source: Hambrey and Fitzsimons, (2010)

**Regelation:** also known as Weertman regelation, named after Johannes Weertman's pioneering research on glacier sliding over bedrock (Weertman, 1957). Glaciers commonly flow over rough beds. Ice flow against a bedrock obstacle causes a pressure increase on the stoss side of the obstacle, which melts the ice. The liquid water travels around the bump following a pressure gradient towards the lee side of the obstacle (Weertman, 1957; Kamb and La Chapelle, 1964; Hubbard and Sharp, 1993). Water refreezes here due to the decreased pressure, and can entrain sediment from the surface of the bedrock obstacle. Latent heat of fusion, generated during freezing, is conducted back through the bedrock bump to promote further melting on the stoss side of the obstacle (Fig 1.7). This process is particularly effective around obstacles with a length below 1 m, due to the conduction of latent heat (Weertman,

1957). Basal ice produced by this mechanism typically has a layered appearance with laminations of debris-poor and debris-rich ice on the order of mm or less in thickness.



**Figure 1.7. Weertman regelation.** Ice melts on the stoss side of the obstacle and travels as liquid water around it until pressure decreases enough to allow re-freezing on the lee side. Heat liberated from this process is conducted through the obstacle enhancing melting.

**Freeze-on by conductive cooling:** Water is commonly found beneath glaciers, especially during the spring-summer melt season (Hubbard and Nienow, 1997; Irvine-Fynn et al., 2011). This water can percolate into unconsolidated substrates below the glacier and into certain types of bedrock, such as limestone. Water can freeze subglacially when the 0°C isotherm reaches the substrate (particularly during winter), which entrains debris onto the glacier base (Weertman, 1961). This process is commonly associated with the formation of sediment-rich 'stratified facies' basal ice (Knight, 1997).

**Supercooling:** the melting/freezing point of water depends on the ambient pressure. In glaciers, changes in this melting/freezing point can vary under different situations.

1) Water remains liquid when adhered to sediment grains at sub-zero temperatures, due to interfacial effects. Additionally, ice crystal growth is inhibited due to lack of space between the grains. This has been reported to occur in ice streams in Antarctica, where water may migrate following pressure gradients through pores and veins in the ice to a freezing front at the ice-bed interface. (Christoffersen, 2003; Christoffersen et al., 2006). The presence of this liquid water around sediment grains entrained in ice is important in relation to supporting life in ice and, as such, has been

suggested to be a “hotspot” for life where metabolically active microbes can thrive (Tung et al., 2006; Price, 2007).

2) Glaciohydraulic supercooling occurs when the pressure melting point of water, ascending the adverse slope of a subglacial overdeepening, rises faster than the water is heated by viscous dissipation. Due to pressure effects associated with thicker ice, the melting point of water is depressed in the deepest part of the basin. The water is forced out, following a pressure gradient, towards the edge of the glacier. If not heated sufficiently by viscous dissipation, water may reach the margin at atmospheric pressure at a temperature below 0°C (i.e. is supercooled). For supercooling to occur, the adverse slope of the overdeepening must be 20-70% steeper than the ice-surface slope (Alley et al., 1998; Lawson et al., 1998; Cook et al., 2006). In order to maintain thermal equilibrium with its surroundings, some of the water will freeze to release latent heat. This freezing produces unique ice types: frazil ice and anchor ice. Certain types of sediment-rich (stratified facies) basal ice have been proposed to be formed by this process as they share physical, sedimentological and isotopic similarities with anchor and frazil ice (Alley et al., 1998; Lawson et al., 1998; Cook et al., 2007, 2010).

#### 1.4. Chemistry of basal ice

Most of the ice within glaciers is derived from meteoric deposition of snow that has undergone a process of firnification. Consequently, the chemical composition of glacial ice is similar to the chemical composition of the snow input (Knight, 1997; Bhatia et al., 2006). Near the bed, where the processes that form basal ice operate, the close contact of the ice, meltwater and rock will modify the ice chemistry, enriching ice in certain ions derived from the substrate (Knight, 1997). Previous research showed that basal ice enrichment in ions depends on the bedrock. For example, Alpine glaciers showed higher values of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  derived from limestone bedrock (Hallet et al., 1978). Additionally, the presence of living microorganisms has been reported to affect the chemical composition of basal ice, reducing the concentration of  $\text{O}_2$  and increasing  $\text{CO}_2$  as respiration occurs (Montross et al., 2014). The depletion of  $\text{O}_2$  associated with respiration requires anaerobic respiration in order for microorganisms to respire, enriching basal ice in other

chemical species, such as  $\text{N}_2\text{O}$  (Prisco, 1997). Certain rock types, such as calcite and limestone, mainly composed of  $\text{CaCO}_3$ , are prone to dissolution, and basal ice derived from subglacial processes in the presence of such bedrock is similarly enriched in  $\text{CaCO}_3$  (e.g. Tsanfleuron glacier - Hallet, et al., 1978; Lemmens et al., 1982; Sharp et al., 1990; Fairchild et al., 1994; Hubbard et al., 2003). In addition, subsurface ice from other sources have shown enrichment in different solutes: Antarctic ice from lake Vostock had a higher sulphate concentration (and consequently, lower pH) (Price, 2000), whilst sea ice has higher salts ( $\text{NaCl}$ ) and higher pH values (Price, 2007).

More generally, basal ice has been reported to be richer than englacial ice in divalent cations, such as  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ , and it has been suggested that these ions derive directly from weathering of the bed. On the other hand, the composition of monovalent cations, such as  $\text{Na}^+$  and  $\text{K}^+$  does not vary as much, and it has been proposed that these ions are derived from meteoritic snow (Lorrain & Souchez, 1978). Accordingly, a low value for the  $\frac{(\text{Na}+\text{K})}{(\text{Ca}+\text{Mg})}$  ratio indicates that ice has been in contact with the bedrock for a long period of time (Knight, 1997). Some basal ice compositions compared to glacial ice are shown in Table 1.

The mineralogy of the sediment entrapped in basal ice also has received attention, especially in cores from the Greenland Ice Sheet. Few studies have characterised the mineralogy entrapped in basal ice - Tung et al. (2006) and Yde et al. (2010) showed relative high abundance of smectite, chlorite, pyrite, and pyroxenes. A more extensive review of the chemistry of basal ice is available in the introduction of Chapter 3.





Table 1.1. Comparison between different types of subglacially-derived basal ice from various sources and other types of samples (apron and glacial ice). Concentrations expressed in ppm.

	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	Site	Source
<b>Basal Ice</b>	0.87	1.03	0.74	0.07	1.81	NA	0.28	NA	Victoria Upper Glacier	(Fitzsimons et al., 2008)
<b>Apron</b>	0.7	0.72	0.35	0.04	1.39	NA	0.28	NA	Victoria Upper Glacier	(Fitzsimons et al., 2008)
<b>Englacial</b>	0.9	0.54	0.71	0.09	NA	NA	NA	NA	South Victoria Land	(Hooker et al., 1999)
<b>Basal Ice (clear)</b>	0.67	0.85	0.62	0.08	NA	NA	NA	NA	South Victoria Land	(Hooker et al., 1999)
<b>Basal Ice (clear)</b>	2.52	1.21	1.25	0.24	NA	NA	NA	NA	South Victoria Land	(Hooker et al., 1999)
<b>Basal Ice (clear)</b>	22.04	1.91	1.45	6.31	NA	NA	NA	NA	South Victoria Land	(Hooker et al., 1999)
<b>Englacial</b>	1.5	1	0.8	0.4	3.7	1.6	0.2	0	Rhone Glacier	(Mager et al., 2009)
<b>Basal ice (amber)</b>	6.3	1.5	3	1.2	11.4	5.6	0.6	0.1	Rhone Glacier	(Mager et al., 2009)
<b>Basal ice (stratified)</b>	2.3	1.1	1.5	0.5	4.3	2.3	0.4	0	Rhone Glacier	(Mager et al., 2009)
<b>Englacial</b>	0.8	0.9	4.2	2.6	NA	NA	NA	NA	Glacier de Tsijiore	(Souchez & Lorrain, 1978)
<b>Basal ice (bubbly)</b>	1.4	1.1	4.1	2.7	NA	NA	NA	NA	Glacier de Tsijiore	(Souchez & Lorrain, 1978)
<b>Basal ice (bubble-poor)</b>	2.9	2.4	6.4	3.1	NA	NA	NA	NA	Glacier de Tsijiore	(Souchez & Lorrain, 1978)
<b>Englacial</b>	2.9	1.9	16.6	1.8	NA	NA	NA	NA	Tsanfleuron Glacier	(Hallet et al., 1978)
<b>Basal ice</b>	2.0	2.0	67.5	4.8	NA	NA	NA	NA	Tsanfleuron Glacier	(Hallet et al., 1978)

### 1.5 Microbial diversity in the subglacial environment and basal ice

Prior to the late 1990s, glacier ice in general and sub-glacial ice in particular was considered to be devoid of biological activity (Priscu et al., 1999; Sharp et al., 1999). The initial microbial cell enumeration of subglacial ice had shown elevated number of cells in basal ice as compared to englacial ice ( $10^3$ - $10^5$  vs  $10^1$ - $10^3$  cells ml<sup>-1</sup>, respectively) (Boetius et al., 2015). Microbial activity in subglacial ice has been further implicated in processes such as mineral weathering (Sharp et al., 1999; Yde et al., 2010; Mitchell et al., 2013) and greenhouse gas production (Wadham et al., 2012; Hood et al., 2015). Previous research has shown that, except for photoautotrophs, all metabolic lifestyles are present in subglacial ecosystems (Boetius et al., 2015), although phylogenetic marker rRNA gene sequences affiliated with photosynthetic organisms have been retrieved from subglacial ecosystems (Kaštovská et al., 2007; Hamilton et al., 2013), likely to have been transported from supraglacial environments or having been entrained in the subglacial systems by overriding algal mats (Skidmore et al., 2000). In order to generate an input of energy under the prevailing subglacial conditions, microbial chemolithotrophy must be operating where freshly exposed and colonized reduced mineral can be oxidised, by C fixing chemolithotrophs and this C-fixing activity has been quantified to an upper limit of several micrograms of carbon m<sup>-2</sup> day<sup>-1</sup> (Boetius et al., 2015). Heterotrophic microbes can feed on this primary fixed C or entrapped ancient organic matter from pre-glacial times (Mikucki et al., 2009). However, low carbon content has previously been reported in subglacial ecosystems (Anesio and Laybourn-Parry, 2012; Lawson et al., 2015) and the fraction of bioavailable C is significantly lower (Lawson et al., 2015). As the predominating C input driver is chemolithotrophy, the chemical and geological nature of the bedrock must play a fundamental role in shaping the microbial community inhabiting the subglacial environment (Skidmore et al., 2005; Mitchell et al., 2013). The near total isolation of the subglacial environment from the atmosphere means that in the absence of a supply of subglacial oxygen, local environmental conditions of anoxia will predominate as chemolithotrophy and aerobic heterotrophic metabolisms progress (Anesio and Laybourn-Parry, 2012; Hodson et al., 2015). Under these conditions, anaerobic respiration would be selected for in order to account for microbial growth and production, as evidenced in

the detection of nitrate and sulphate reduction (Skidmore et al., 2000; Boyd et al., 2011). Under these typical anaerobic conditions, methanogenesis will also be selected for, which has been confirmed in the characterisation of sequences associated with methanogenic archaea (Boyd et al., 2010; Dieser et al., 2014) and simulation studies have shown that the subglacial environment might accumulate significant amounts of methane, which would have potentially important implications for climate change following release to the atmosphere (Wadham et al., 2008; Wadham et al., 2012).

Although important and abundant in cold environments, such as glacier forefronts (Zumsteg et al., 2012; Brown and Jumpponen, 2014), there has been limited investigation of fungi in subglacial environments compared to supraglacial environments (Kaczmarek et al., 2016). Basidiomycete yeasts have been identified in some samples of basal ice (D'Elia et al., 2009; Buzzini et al., 2012) with implications for heterotrophic oxidative and fermentative activities, but only fragmented mycelia of filamentous fungi have been reported in basal ice in samples from Lake Vostok in Antarctica (Abyzov et al., 2001). However, certain filamentous fungal genera have been detected in subglacial environments and basal ice, with a high proportion of *Penicillium* genus members (Sonjak et al., 2006).

#### *1.5.1 Microbial metabolism in basal ice*

##### **Chemolithotrophy**

Subglacial environments are dark; hence photosynthesis cannot be supported, despite the fact that some photosynthetic organisms, e.g. cyanobacteria, have been identified in this environment (Kaštovská et al., 2007). Furthermore, basal ice and subglacial environments are oligotrophic environments (Margesin and Miteva, 2011; Montross et al., 2014) that will select for primary chemolithotrophs in order to sustain a microbially active environment. As basal ice is often sediment rich (Hubbard, Bryn and Sharp, 1989; Knight, 1997; Hubbard et al., 2009), key inorganic redox nutrients are available for chemolithotrophic microorganisms. The processes involved in basal ice formation guarantee a supply of freshly exposed mineral entrapped in basal ice that can be utilised by the microbial community (Telling et al., 2015). A plethora of chemical species can be oxidised in order to generate energy in

chemolithotrophic metabolism and include reduced forms of sulphur (S), iron (Fe), hydrogen (H), and nitrogen (N). Different microbe-specific capabilities reflect the heterogeneous mineralogy of bedrock-derived sediment that becomes incorporated in basal ice and that shapes the resulting basal ice microbial community (Mitchell et al., 2013). As a consequence of chemolithotroph-mineral interactions, subsequent oxidation of certain elements (e.g. S) lead to biogenic mineral formation (e.g. jarosite), known to originate as a consequence of biogenic processes (Kawano and Tomita, 2001; Fortin, 2004; Bontognali et al., 2014). Chemolithotrophic-related sequences retrieved from subglacial environments comprise taxa such as *Candidatus Nitrotoga*, able to oxidise N, S, and Fe compounds (Christner et al., 2014), *Thiomicrospira*, a S oxidiser (Boyd et al., 2014), and *Thiobacillus*, *Acidithiobacillus* and *Synderooxidans* (Mitchell et al., 2013) able to oxidise Fe and S.

The presence of methane has been confirmed by gas chromatography in cores that reached the subglacial environments of the Greenland Ice Sheet (Boyd et al., 2010), together with analysis of meltwater outflow from subglacial environments (Dieser et al., 2014). Methanogenic archaea have been identified using fluorescence microscopy at 420 nm (wavelength at which the coenzyme F420, implicated in methanogenesis, emits) (Boyd et al., 2010) and DNA targeting phylogenetic (16S rDNA) and functional (*mcrA*) markers (Boyd et al. 2010; Stibal, Telling, et al. 2012; Wadham et al. 2012). It should be noted that methane can, however, originate from geochemical reactions, but microcosm experiments have confirmed that 90% of the total methane found in subglacial environments proceeded from biological reactions (Boyd et al., 2010; Stibal, Hasan, et al., 2012; Dieser et al., 2014). Chemolithotrophic-associated genera that have been found in subglacial environments are summarised in Table 2.

**Table 1.2. Examples of chemolithotrophic genera identified in subglacial environments**

<b>Metabolism</b>	<b>Example</b>	<b>Reference</b>
<b>Iron oxidisers</b>	<i>Gallionella</i> , <i>Sideroxydans</i>	(Boyd et al., 2014)
<b>Sulphur oxidisers</b>	<i>Acidithiobacillus</i> , <i>Thiobacillus</i>	(Boyd et al., 2014)
<b>Hydrogenotrophs</b>	<i>Rhodoferax</i>	(Hamilton et al., 2013)
<b>Methanogens</b>	<i>Methanosarcinales</i> , <i>Methanomicrobiales</i>	(Boyd et al., 2010; Telling et al., 2012)
<b>Ammonia oxidisers (AOB)</b>	<i>Nitrosomonas</i> , <i>Nitrospira</i>	(Boyd et al., 2011, 2014)
<b>Nitrite oxidisers (NOB)</b>	<i>Candidatus Nitrotoga</i>	(Achberger et al., 2016)

## **Heterotrophy**

Heterotrophic metabolism is characterised by the consumption of organic matter to generate energy. In order to gain energy, heterotrophs use respiratory processes, which can be aerobic, using O<sub>2</sub> as a terminal electron acceptor (TEA), or other oxidised chemical species (Table 1.3). The progression of aerobic respiration in closed systems can lead to the creation of anoxic pockets within the ice (Boyd et al., 2010), which has been reported in proglacial environments (Wadham et al., 2007). Heterotrophic consumption and depletion of oxygen by aerobic bacteria and fungi in closed systems triggers facultative and eventual obligate anaerobic respiration generating non-CO<sub>2</sub> final products, e.g. Fe(II) by Fe-reducers (Nixon et al., 2017). Anaerobic respiration is important for biogeochemical cycling within the subglacial environment (Hamilton et al., 2013). In addition, the presence of certain fungi will also affect biogeochemical cycling, accumulating CO<sub>2</sub> by aerobic respiration and fermentation. Some of the genera described in the previous studies, such as *Penicillium* (Sonjak et al., 2006), can also solubilise phosphate (Li et al., 2016), which is one of the limiting elements for microbial growth (Mohammadi, 2012).

Apart from respiratory assimilation, microorganisms (including bacteria and fungi) inhabiting basal ice can partially oxidise organic matter under oxygen limiting

conditions, in processes of fermentation, which will generate H<sub>2</sub> and CO<sub>2</sub> as by-products, as well as alcohols and organic acids (Christner et al., 2012). These key microbial by-products are central in mineral weathering processes (Uroz et al., 2009) and serve as substrates for methanogenesis (Wadham et al., 2012; Stibal, Wadham, et al., 2012; Dieser et al., 2014). Methane oxidation by methylotrophs and methanotrophs maintains tight C cycling and has been previously reported in subglacial environments (Dieser et al., 2014).

**Table 1.3. Examples of heterotrophic genera identified in subglacial environments**

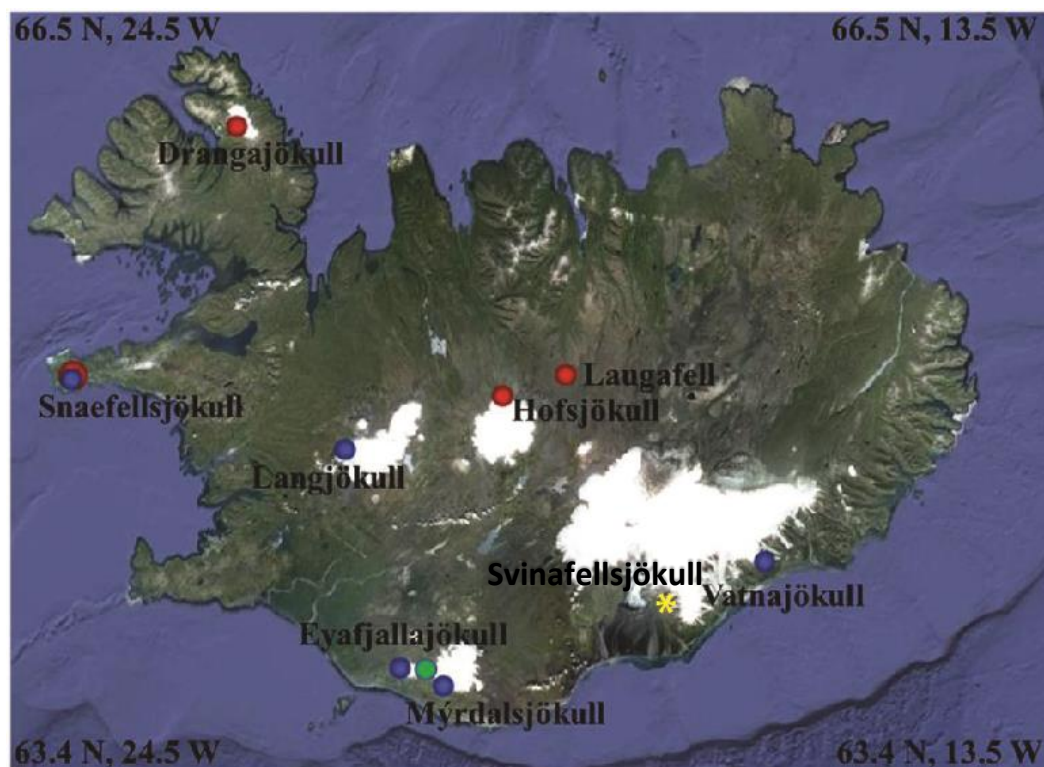
<b>Respiration</b>	<b>Example</b>	<b>Reference</b>
<b>Aerobic</b>	<i>Bacillus, Pyschrobacter, Paenobacillus, Penicillium</i>	Doyle et al., 2013; Kaczmarek et al., 2016
<b>Methanotrophs</b>	<i>Methylobacter</i>	Dieser et al., 2014
<b>N-reducers</b>	<i>Polaromonas</i>	Boyd et al., 2011
<b>S-reducers</b>	<i>Desulfocapsa, Geopsychrobacter, Desulfolobus</i>	Mikucki and Priscu, 2007; Hindshaw et al., 2016
<b>Mn-reducers</b>	<i>Geopsychrobacter</i>	Mikucki and Priscu, 2007)
<b>Fe-reducers</b>	<i>Geothrix, Rhodoferax, Geobacter</i>	Marteinsson et al., 2013; Hindshaw et al., 2016; Nixon et al., 2017
<b>Fermentation</b>	<i>Clostridium</i>	Stibal et al., 2012

## 1.6 Microbial diversity in Icelandic glacial and periglacial environments

Icelandic glaciers represent a special case among glacier systems due to their close proximity to active volcanoes, which provide a frequent input of volcanic ash (Lutz et al., 2015) and ash-derived minerals such as N, P, Fe (Ritter, 2007; Jones and Gislason, 2008). In addition, some Icelandic glaciers have developed directly over active volcanoes providing direct input of geothermal energy and volcanic-derived minerals (Marteinsson et al., 2013). The presence of ash on the ice surface can enhance ice melt through reductions of snow and ice albedo, absorbing light and localised heating within the ash deposit (Lutz et al., 2015).

Lutz et al. (2015) reported on the microbial diversity of surface ice/snow in a survey of Icelandic glaciers (Fig 1.8). The most abundant phyla were *Alphaproteobacteria* at

Drangajökull and Vatnajökull, *Betaproteobacteria* at Langajökull, Laugafell, Snaefellsjökull, and Eyjafjallajökull and *Saprospirae* at Hofsjökull and in certain samples from Eyjafjallajökull. A limited diversity of archaeal sequences from the same glaciers were identified. *Nitrososphaerales* was the dominant order in most of the glaciers (Laugafell, Vatnajökull, Langjökull, Snaefesjökull) except for Hofsjökull, where *Methanosarcinales* dominated the archaeal community. Following the Eyjafjallajökull eruption in 2010, primary colonisation in the Fimmvörðuháls basaltic lavas was dominated by *Betaproteobacteria*, some its members affiliated with chemolithotrophs, heterotrophs and diazotrophs and negligible representation of phototrophs (Kelly et al., 2014). Analysis of subglacial lakes beneath the Vatnajökull icecap showed that the Bacterial communities were dominated by *Firmicutes* (*Acetobacterium*), with a high abundance of lake-specific *Thermus-Deinococcus* (*Thermus*) and *Bacteroidetes* (*Paludibacter*) or *Deltaproteobacteria* (*Geobacter*) in the two lakes sampled (Marteinsson et al., 2013)



**Figure 1.8. Location of Iceland's glaciers and icecaps.** Glaciers analysed in Lutz et al. (2015) are represented as coloured dots: red, glaciers analysed in 2012, blue, 2013, and 2015, green. The location of Svínafellsjökull, the main research site of this thesis, is represented as yellow star. Map adapted from Lutz et al. (2015).

## 1.7 Glaciers studied in this thesis

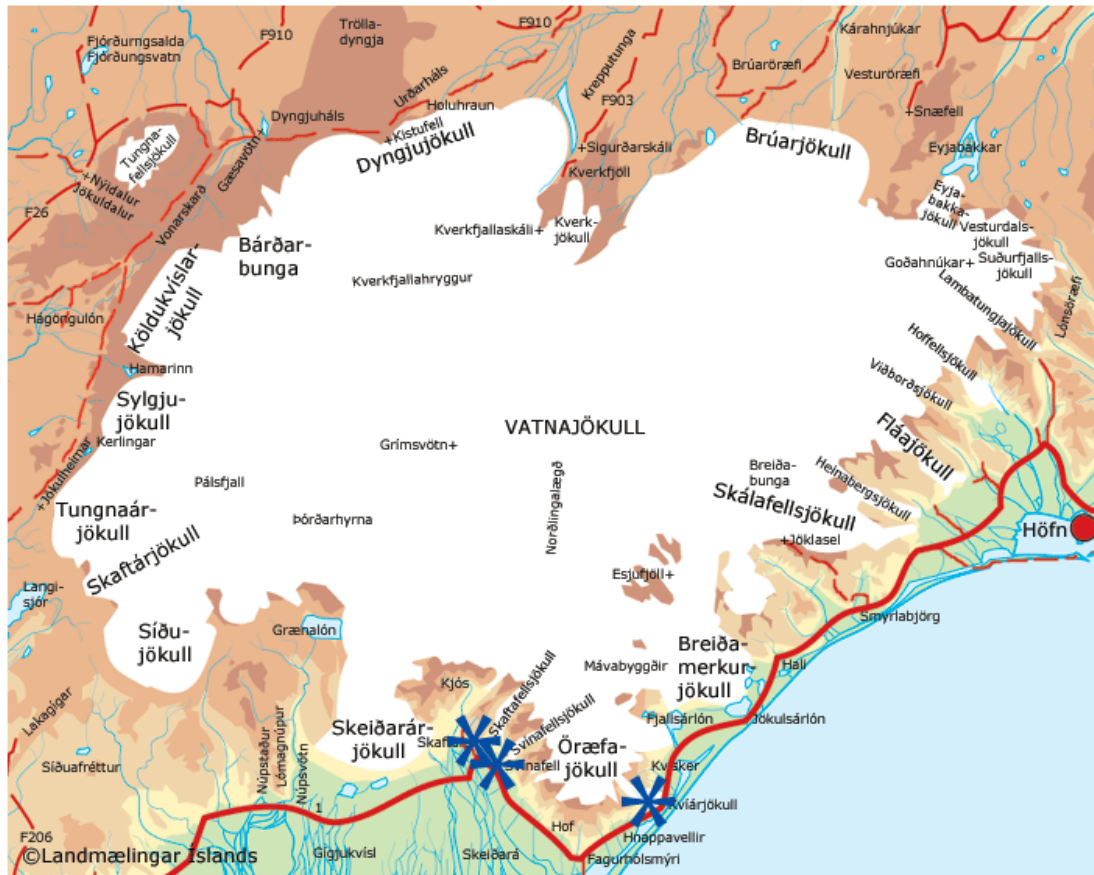
### 1.7.1 *Svínafellsjökull*

#### 1.7.1.1 Background to the glacier

Svínafellsjökull (63°59' N, 16°52' W) is an outlet valley glacier from the Öræfajökull icecap (Fig 1.9), where Hvannadalshnúkur, the highest peak in Iceland, is situated (2110 m.a.s.l.). Öræfajökull is joined to the Vatnajökull icecap in the northern sector. Svínafellsjökull is situated in the Skaftafell region delimited by Skaftafellsjökull glacier and the Hafrafell mountain to the north-west and with Virkisjökull to the south (Fig 1.9). The Skaftafell region has a maritime climate with cool summers and mild winters, with a mean annual temperature over the period between 1996-2007 of ~5°C, averaging 10.5 °C in July and 3.3 °C in January. The annual precipitation over the period between 1949-2007 logged at the nearby climate station of Fagurhólsmýri is 1800 mm (Vilmundardóttir et al., 2014).

Svínafellsjökull has a total ice volume of 3.6 km<sup>3</sup> spread across 33.2 km<sup>2</sup> and is 6 km in length (Hannesdóttir et al., 2015). Svínafellsjökull flows from an icefall situated in the easternmost part of the glacier tongue, at an elevation of 2030 m.a.s.l, towards the west. The glacier terminates in a overdeepening at a height of 90 m.a.s.l.





**Figure 1.9. Outlet glaciers from the Vatnajökull and Öraefajökull ice caps.** Glaciers investigated in this thesis (Skaftafellsjökull, Svínafellsjökull, and Kvíarjökull) are marked as blue stars. Source [www.nat.is](http://www.nat.is)

#### 1.7.1.2 Foreland

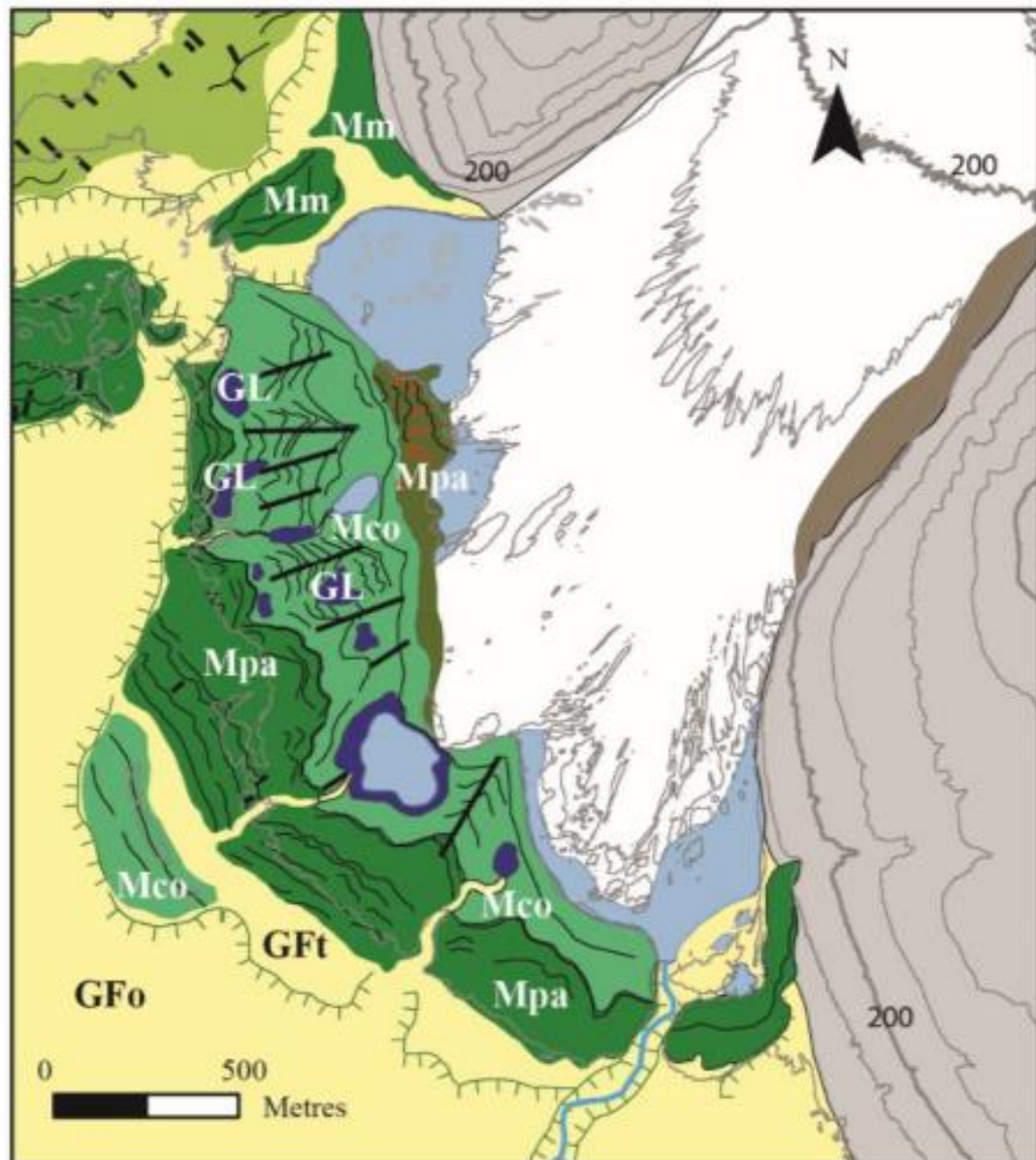
The foreland of Svínafellsjökull extends for approximately 1 km between the glacier terminus and the most distal moraine (Fig 1.10).

Starting from the glacier margin, the first sets of moraines that can be identified are seasonal push moraines, in some cases in direct contact with the ice margin (Fig 1.10). The height of these moraines varies between 0.3 to 1.5 m. The sediment forming these moraines consists of a clay matrix with poorly sorted grains of highly variable sizes (Lee, 2016).

At ~500m from the ice margin, two sets of arcuate moraines can be identified in the north side of Svínafellsjökull (Fig 1.10), one dating from Little Ice Age (LIA) (maximum dated locally to 1870-1890 AD) and one dating from 1935. They are mainly composed of basalt and hyaloclastite (Lee, 2016). The height of these moraines ranges from 0.65 m to 7.2 m. Where the snout of the glacier was situated in this location, Svínafellsjökull and Skaftafellsjökull were conjoined into a single piedmont glacier

lobe (Fig 1.11). On the southernmost side of the glacier only one set of arcuate moraine are featured (Lee, 2016) (Fig 1.10).

The most distant and more predominant moraine is a composite moraine (Fig 1.10), known as Storalda moraine that is completely vegetated, with a height of 22 m, situated ~1 km from the glacier terminus (Lee, 2016). Using lichenometric dating techniques, Storalda moraine has an estimated age of 2500 years (Gonzalez et al., 1999; Guðmundsson et al., 2002). This moraine represents the distal boundary of the Svínafellsjökull foreland.



#### A Landsystem Element Symbology

<b>Moraine</b>	<b>Glaciofluvial</b>	
Overridden Moraine	Outwash	River
Arcuate Push Moraine	<b>Other Features</b>	Terraces
Sawtooth Push Moraine	Scree	Moraine Crest
Medial Moraine	Mountain	Elevation (m asl)
Low Amplitude Push Moraine	Glacier	200 m contour interval
<b>Glaciolacustrine</b>	Lake	Streamlined Feature
Lacustrine		Crevasse Squeeze Ridge

Mco	Composite Moraine
Mpa	Push moraine
Mm	Medial Moraine
GL	Glaciolacustrine
GF	Glaciofluvial

**Figure 1.10. Geomorphological features in the forefield of Svínafellsjökull, including proglacial lakes, moraines, and outwash plain. Adapted from (Lee, 2016)**

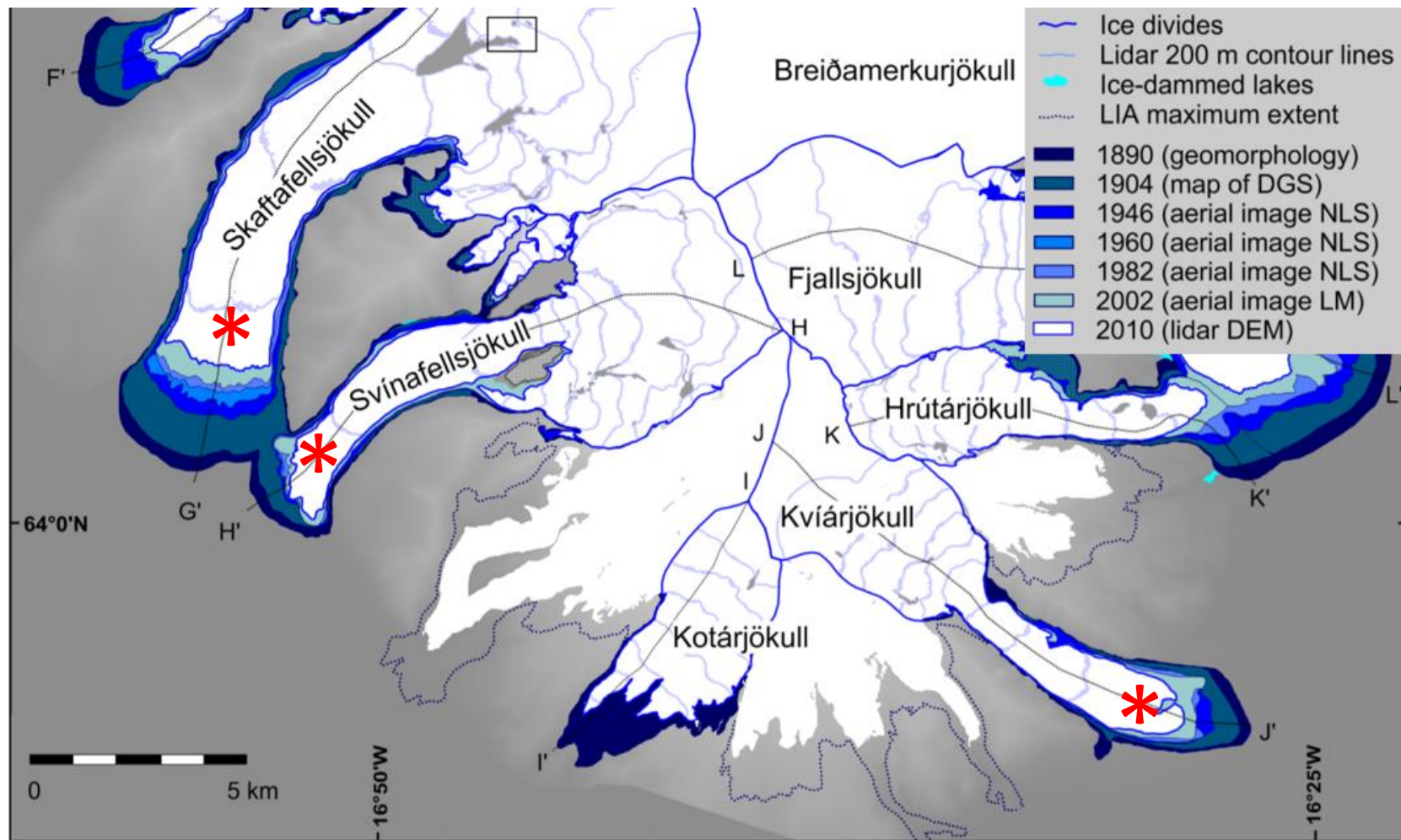
#### 1.7.1.3 Glacier terminus dynamics

The Skaftafell region in which Svínafellsjökull is situated, has undergone at least 20 glacial intervals in the last 9 Ma (Eiríksson et al., 1990; Eiríksson and Geirsdóttir, 1991; Eiríksson, 1994). Previous studies have suggested the region has been permanently covered with ice since late Miocene time (5.3 Ma) (Eiríksson, 1994) or Brunhes time (0.78 Ma) (Helgason and Duncan, 2001).

Svínafellsjökull's terminal position and thickness has varied over time in response to climatic shifts. Based on historical records, Ives (2007) reported that Svínafellsjökull's terminus position was 3.5 km further up-valley in the 14<sup>th</sup> century and the foreland of the glacier was used for agriculture by early settlers. So, between the 1400s and the Little Ice Age, Svínafellsjökull advanced and overrode this agricultural soil, which is likely to constitute part of the current subglacial debris load.

Since 1995, Svínafellsjökull as all glaciers in the Vatnajökull region (Schomacker, 2010), has exhibited a trend of net recession and thinning. However, the rate of retreat at Svínafellsjökull is lower than the rates of other glaciers in the region. It has been estimated that the Vatnajökull ice cap could lose 25% of its volume by 2050, and might disappear completely in 200 years (Schomacker, 2010). Since the LIA, Svínafellsjökull has receded into its terminal overdeepened basin, but retreat rates vary along the glacier margin: 500 m in the southern margin, and 1000 m in the northern sector (Lee, 2016). Since the LIA, Svínafellsjökull has receded on average ~800 m into its subglacial basin, which corresponds to a loss of ~ 20% of the glacier's total LIA volume, equivalent to 458 km<sup>3</sup> of water (Bradwell et al., 2013; Schmidt et al., 2013; Kirkbride, 2002; Lee, 2016) (Fig 1.11), with two short periods of advance in 1904 and 1932 (Þórarinnsson, 1956; Thompson and Jones, 1986).

In the last 10-15 years, proglacial water bodies have proliferated in the foreland due to glacier recession into the terminal basin (Cook et al. 2011b). This process has been also found in some other glaciers in the region, and in other locations globally (Schomacker, 2010; Cook and Quincey, 2015) (Fig 1.11). The formation of lakes at the termini of Icelandic glaciers has been highlighted as an important process for sediment trapping, preventing the transport of the aforementioned sediment to the outwash plains and eventually to the coast (Schomacker, 2010).

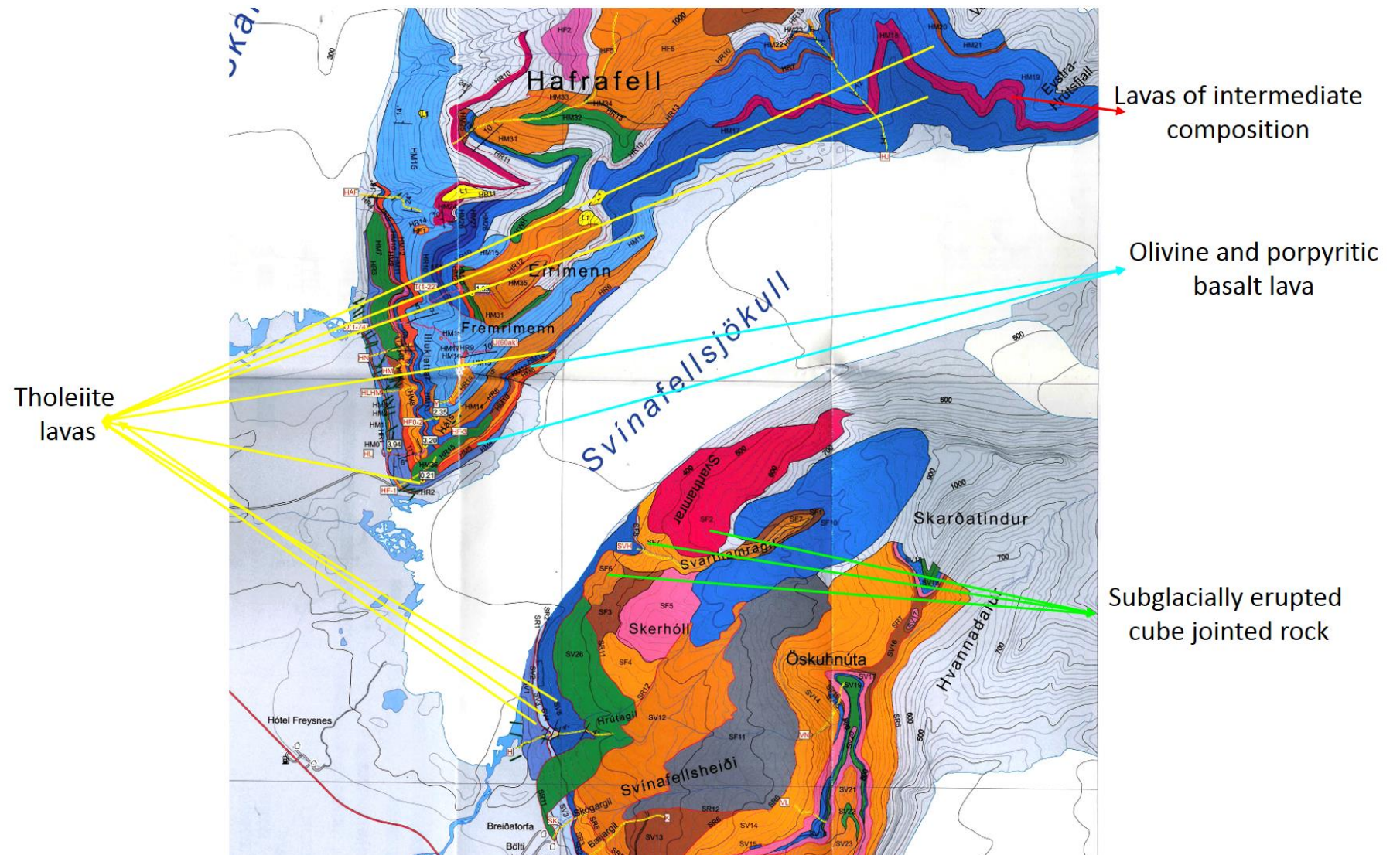


**Figure 1.11.** Recession of glaciers from Öræfajökull and Vatnajökull icecaps since LIA based on aerial images and geomorphology studies. Glaciers analysed in this work are marked with red stars. Adapted from Hannesdóttir et al., 2015

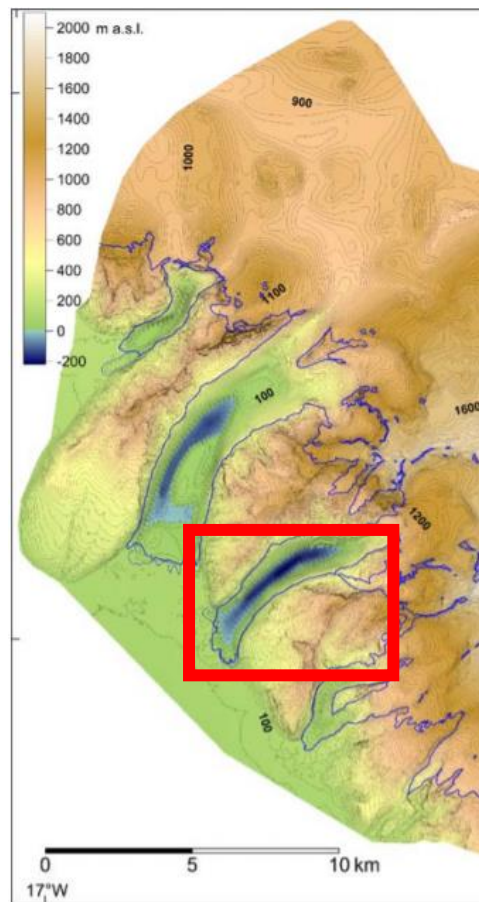


#### 1.7.1.4 Geological setting

The Öræfajökull icecap rests on top of the volcano of the same name, and hence the bedrock that underlies Svínafellsjökull is dominated by basalt, hyaloclastite, and subglacially erupted cube jointed rock (Fig 1.12). Öræfajökull is an active volcano located at the southern end of a so-called flank zone, 50 km east of the eastern axial rift zone in Iceland, where the European and North American tectonic plates separate (Prestvik et al., 2001). Glaciers in this region have carved the volcanic strata, generating valleys (Fig 1.13). Helgason and Duncan (2001) proposed that, at around 4 Ma, lavas accumulated in the region, showing no evident signs of erosion. Between 3-4 Ma some erosional processes were observed between the lava flows, and between 3-0.8 Ma there are evident signs of glacial erosion. Results by Helgason and Duncan (2001) suggest that erosive processes have been most efficient than volcanic-derived rock formation processes in the Skaftafell area in the last 800,000 years, leading to the formation of deep eroded valley glaciers.



**Figure 1.12. Geology of the bedrock at Svínafellsjökull.** In grey sedimentary rock and tillite, and in colour different volcanic origin geological units. The map has been manually annotated to show the most relevant geological formations in contact with the glacier.



**Figure 1.13. Bedrock topography of the glaciers outflowing from the western side of Öræfajökull ice cap.** The contour lines were drawn at 20 m intervals. In blue, bedrock below the sea level. Blue lines show limits of the current glaciers. The red square demarcates the Svínafellsjökull glacier. Adapted from (Magnússon et al., 2012).

In their study, Helgason and Duncan (2001) found that the region within which Svínafellsjökull sits showed evidence of both subaerial eruptions (coarse-grained olivine tholeiites, fine-grained aphyric tholeiites, plagioclase porphyritic units and thicker fine-grained aphyric basaltic andesites) and subglacial eruptions (lobes, pillows, pillow breccia, hyaloclastite breccia and hyaloclastite), intercalated with glaciofluvially worked sediments. In general, rocks from the Skaftafell area more alkaline than average in Iceland, and higher in K.

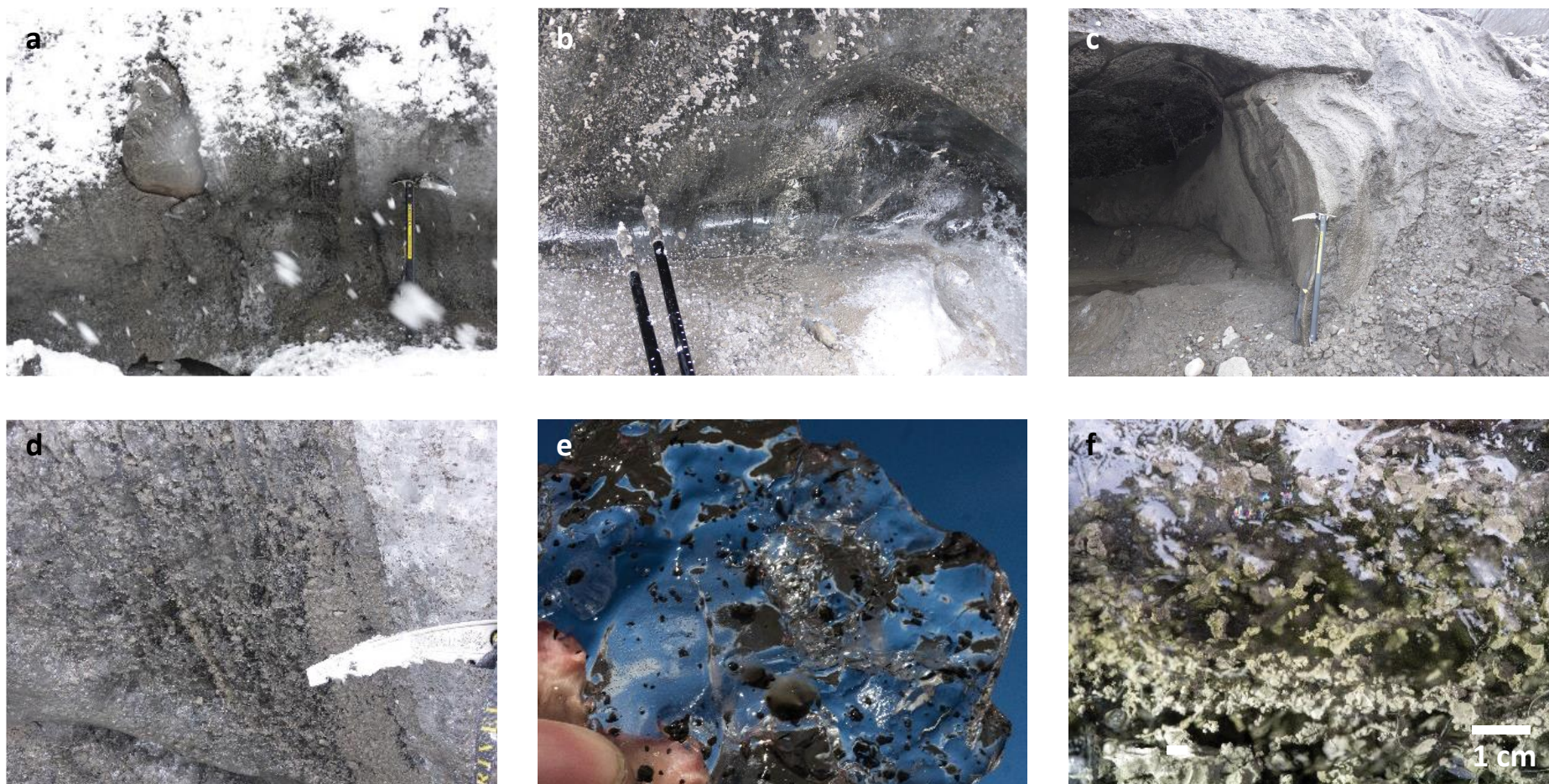
Figure 1.12 shows a map of the bedrock within the immediate area. The northern side of the Svínafellsjökull adjacent to tillite, next to tholeiite basalt lavas, olivine and porphyrite basalt lavas, and some subglacially erupted lavas. The bedrock on the southern side of the glacier is mainly formed by tholeiite lavas, followed by subglacially erupted cube jointed rock and tillite.



#### 1.7.1.5 Basal ice classification at Svínafellsjökull

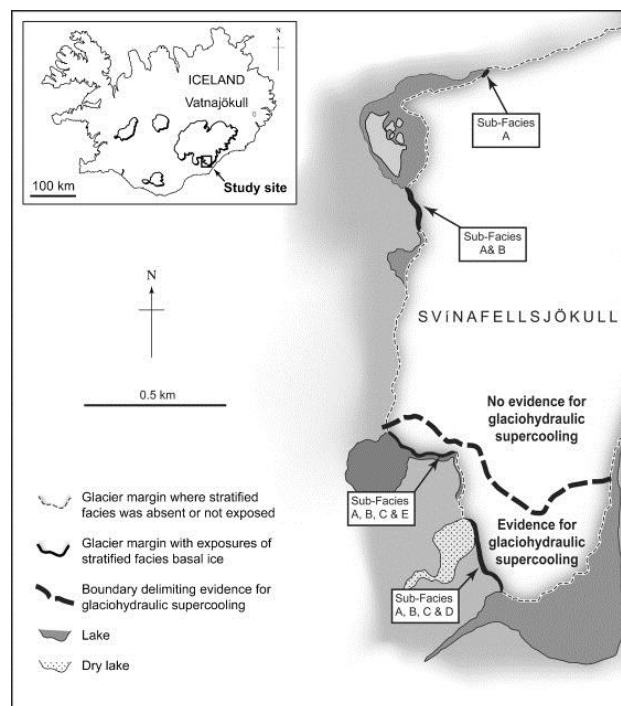
Cook et al. (2007, 2010) described at least three basal ice types or facies at Svínafellsjökull, namely, debris bands (B), dispersed (D) and stratified (S). These ice types have been hypothesised as having different origins and differ in their physical properties and appearances.

Both debris bands and dispersed facies ice have been hypothesised to develop, initially at least, beneath icefalls (Swift et al., 2006; Cook et al., 2011b). The presence of ogives (dark and light banded patterning on ice surface of the glacier) immediately down-glacier of the icefall has been suggested to be indicative of a sharp velocity decrease at the base of the icefall where intense longitudinal compression takes place. Under these compressional conditions, debris-laden basal ice and subglacial sediment can be folded tightly and elevated upward into the body of the glacier, from which shear planes can develop, potentially elevating debris to the glacier surface where they outcrop as dark ogive bands (Fig. 1.5) (Goodsell et al. 2002; Swift et al., 2006; Cook et al. 2011b). A similar process, often termed 'englacial thrusting', may occur in the presence of a terminal subglacial overdeepening, which is also present at Svínafellsjökull. Here, intense longitudinal compression develops as a consequence of glacier flow against the reverse bedslope of the overdeepening, which elevates debris bands into englacial and supraglacial locations. Debris bands (Fig 1.14 a and d) are characterised by their high sediment content and polymodal sediment particle size distribution (Swift et al. 2006). At Svínafellsjökull, debris bands are found at locations in many parts of the glacier margin (Swift et al., in revision). At Svínafellsjökull, Cook et al. (2011b) hypothesised that the basal part of ogive debris bands become metamorphosed through strain deformation and regelation to produce dispersed facies. Dispersed facies has much lower sediment content (1.6%), appears in metre- to tens of metres-thicknesses, and appears pervasively all along the Svínafellsjökull glacier margin (Fig 1.14 b, e) (Cook et al., 2011b).



**Figure 1.14. Basal ice types identified at Svínafellsjökull.** Top row shows general shots of the basal ice samples *in situ* before sluicing and/or cleaning. Bottom row shows close-up images of the different faces. A and D shows debris band, B and E shows dispersed facies, and C and F shows stratified facies. In order to give a sense of scale ice axes (a, c, and d), walking poles (b), and fingers (e) were photographed next to the different basal ice facies.

Stratified facies is typically thought to form through regelation, basal adfreezing (migration of the freezing front into water saturated subglacial sediment (Hubbard, 1991)) close to the glacier margin (Knight, 1997), and/or glaciohydraulic supercooling. At Svínafellsjökull, Cook et al. (2007, 2010) found evidence for a population of basal ice thought to be produced by regelation (so-called 'sub-facies A and B' – Fig. 1.15), and a separate population formed by supercooling (which they named 'Sub-facies C, D and E' – Fig 1.15). The supercooling-related ice facies were concentrated in the southern part of the glacier terminus, where it is thought that there is a subglacial overdeepening, and hence where conditions for supercooling are met (see Section 1.3), and where there was evidence for supercooling in the form of upwellings with anchor and frazil ice terraces. In this study, only the supercooling-related stratified ice was sampled, as this was the only stratified ice observed during field campaigns. Stratified basal ice is characterised by its higher sediment concentration (~30-35% sediment on average), layered appearance, strong representation of silt and fine sand, and inclusion of apparently water-worked, as well as subglacially worked, clasts (Cook et al., 2007, 2010, 2011b) (Fig 1.14 c, f).



**Figure 1.15. Location of sampled glacier basal ice types along the margin of Svínafellsjökull and the area where evidence of glaciohydraulic supercooling was identified in 2004-05** Facies A) Regelation-related stratified ice facies, B) Regelation-related stratified ice facies, C) Supercooling-related ice facies, D) Supercooling-related ice facies, E) Supercooling-related ice facies Taken from Cook et al., (2007)

### *1.7.2 Skaftafellsjökull*

Skaftafellsjökull (64° 01' N, 16° 56' W) is a temperate glacier (Larson et al., 2010) sourcing primarily from Vatnajökull with a minor component at its eastern margin derived from Örfajökull (Tweed et al., 2004; Cook et al., 2010). It limits with Morsárjökull on its western side and Svínafellsjökull on its eastern side separated by the Hafrafell mountain (Fig 1.9). The climate is maritime, with cool summers and mild winters, with a mean temperature of 5.1°C, spanning from 10.5 °C in July to 3.3°C in January (Vilmundardóttir et al., 2014). The geology of the bedrock is dominated by basalt, hyaloclastite and tephra (Vilmundardóttir et al., 2014).

Skaftafellsjökull extends ~20 km (Evans et al., 2017) and the terminus of the glacier is ~120 m.a.s.l. and it is ~3 km wide, connected to a coastal plain that extends from Skeiðarárjökull to Oræfi (Marren and Toomath, 2013). It occupies a total area of 84.1 km<sup>2</sup> and has a volume of 20.3 km<sup>3</sup> (Hannesdóttir et al., 2015). As other Icelandic glaciers, Skaftafellsjökull has experienced a significant recession, ~1.8 km since the LIA (Hannesdóttir et al., 2015), evidenced by the presence of a well-developed morainic system (Marren and Toomath, 2013). Only minor advances in Skaftafellsjökull have been documented in the early 1950s, 1957, and 1968, and the glacier loss has been estimated to be of 1393 m in 2010 (Marren and Toomath, 2013). The recession linked to the presence of a subglacial basin has led to the formation of proglacial lakes (Marren and Toomath, 2013).

The presence of basal ice in Skaftafellsjökull has been previously reported. Glaciohydraulic supercooling stratified facies has been identified, associated with the presence of a subglacial basin (Cook et al., 2007, 2010).

### *1.7.3 Kvíarjökull*

Kvíarjökull (63° 56' N, 16° 27') is a temperate outlet glacier of the Örfajökull icecap (Larson (Swift et al., 2006; Larson et al., 2010). Kvíarjökull is situated on the eastern part of the icecap and derives from an icefall situated at 2010 m.a.s.l. and terminating over a basin at 30 m.a.s.l. (Swift et al., 2006; Hannesdóttir et al., 2015). It extends over an area of 23.2 km<sup>2</sup> with a total volume of 4.1 km<sup>3</sup> (Hannesdóttir et al., 2015). Kvíarjökull presents an over-deepened subglacial basin (Larson et al., 2010). Similarly



to Svínafellsjökull and Skaftafellsjökull, Kvíarjökull is situated over a bedrock formed by volcanic rock, mainly basalt (Larson et al., 2010).

Kvíarjökull has experienced recession since the LIA, as the presence of a very prominent moraine in the foreland testifies (Swift et al., 2006) and has been estimated to be of 1.5 km (Hannesdóttir et al., 2015; Evans et al., 2017) into its over-deepened basin (Larson et al., 2010) and a proglacial lake is developing in front of the glacier margin (Bennett and Evans, 2012).

Basal ice has been reported in Kvíarjökull previously, and glaciohydraulic supercooling stratified facies have been identified around the margin (Larson et al., 2010), as well as subglacially-derived debris bands (Swift et al., 2006).

### 1.8 Aims and objectives

This study aims to characterise the geochemical composition and microbial diversity of basal ice at Svínafellsjökull, Iceland. Using a variety of chemical techniques, the PhD investigates how the chemistry affects, and is affected by, the presence of active microorganisms within the basal ice. The microbial diversity of the basal ice layer was characterised by Next Generation Sequencing (NGS) methodologies, using the bacterial 16S rDNA gene and the fungal Internal Transcribed Spacer (ITS) sequences. In order to have an estimate of the viability and diversity of viable cells in basal ice, culturing methods were used, namely spread plating and isolation chips (ichips). The PhD assesses the microbiome associated with basal ice, and sheds light on the processes operating in the subglacial environment.

### Objectives and realisation

**CHAPTER 3** aims to characterise the geochemical nature of the individual basal ice facies. Questions to be examined include:

- A. Characterise the chemical composition of the basal ice layer at Svínafellsjökull
- B. If different processes generate specific basal ice facies, do the aforementioned processes leave a distinctive chemical imprint in the ice types?

- C. Is the geochemistry of the basal suitable for microbial proliferation?

**CHAPTER 4** will investigate the diversity of the microbial community inhabiting the basal ice using two phylogenetic markers: 16S rDNA for bacteria, and ITS for fungi. In doing so, a snapshot of the ecosystem can be generated allowing the following questions to be addressed:

- A. Is there a microbial community inhabiting basal ice? How abundant are cells in the basal ice attached to the sediment and in the ice matrix?
- B. What bacterial and fungal phyla/genera inhabit basal ice?
- C. Is there any functional relationship between the microorganisms inhabiting basal ice?

**CHAPTER 5** will calculate the amount of microorganisms being transferred from the basal ice layer to the glacier margin based on previous work that estimated inorganic sediment transfer (Cook et al., 2010; Cook et al., 2011b). To do so, cell concentration associated with sediment was calculated using fluorescence microscopy. This chapter will aim to address the following questions:

- A. Is Svínafellsjökull transporting cells and releasing them to the margin in its flow?
- B. If there is cell transport associated with glacial flow, are there any viable cells?

**CHAPTER 6** aims to characterise active and viable microbiota inhabiting basal ice using traditional and innovative cultivation methodologies. The unique opportunity to resolve isolates up to species level by 16S rDNA sequencing permits the identification of the sources where the most similar individuals were isolated. In order to assess the potential origin and fate of the microbiota of basal ice, samples from supraglacial, subglacial sediment, and proglacial soils were cultivated. The questions to be addressed in this chapter will be:

- A. How abundant are viable bacteria in the basal ice layer, supraglacial and subglacial sediment, and proglacial soil?
- B. How active are basal ice and proglacial microorganisms at cold temperatures?
- C. What are the potential origin and fate of the microorganisms entrapped in basal ice?

**CHAPTER 7** sums up and integrates all the data generated in the PhD. The main three topics that this synthesis will cover are: a) how the geochemistry demonstrates that basal ice is an environment suitable to be inhabited by an active microbiota; b) the potential relationships between the microbiota and the minerals identified in basal ice; and c) the potential for delivery of microbiota from glaciers into proglacial environments, and the potential importance of this “microbial conveyor belt” effect. The last section of this chapter will consist of concluding remarks and potential future work based on the results acquired in this PhD.

## 2. Methodology

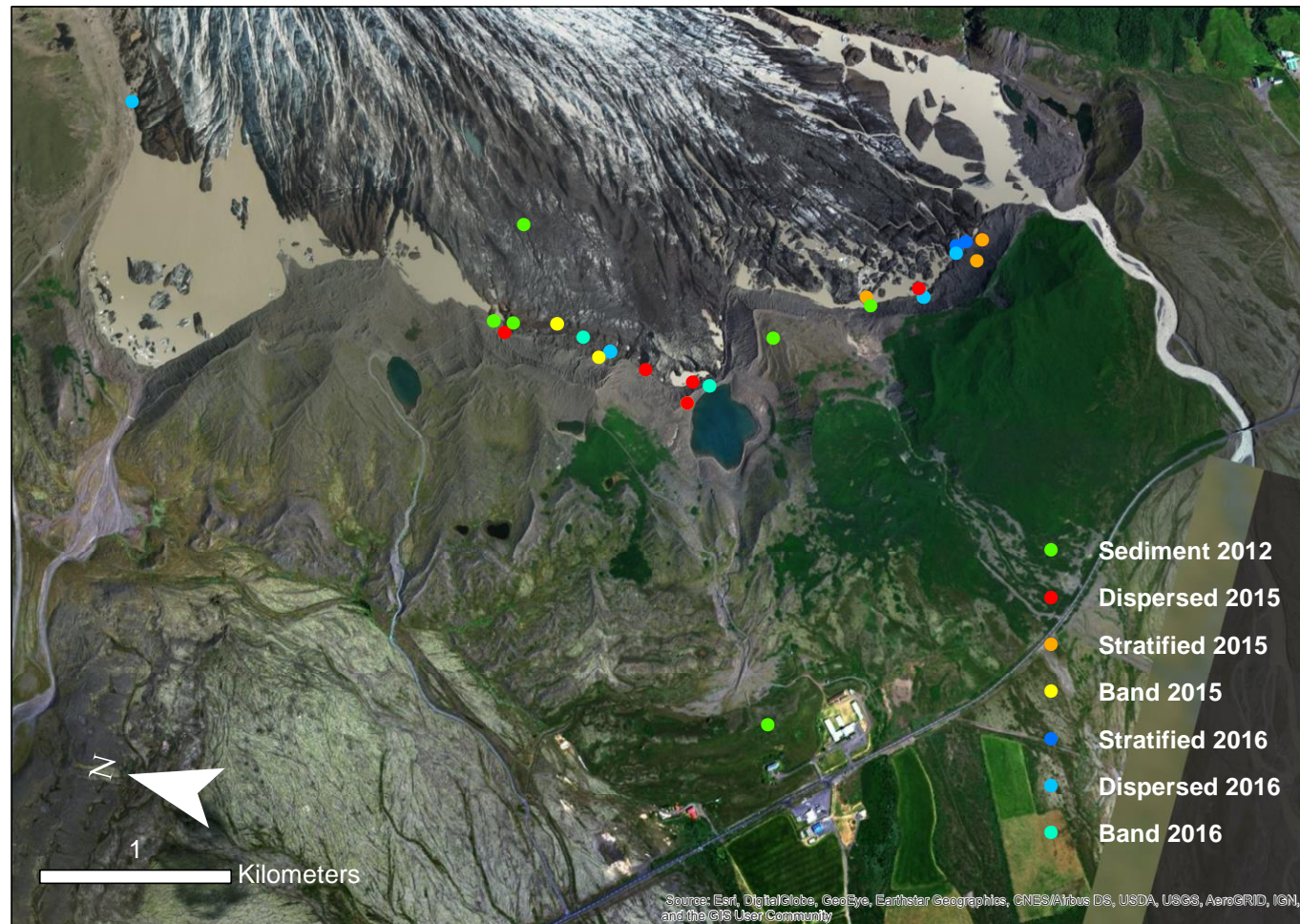
The aim of this chapter is to describe the sampling procedure utilised for the extraction of samples from Svínafellsjökull (Fig 2.1), but also from two neighbouring glaciers: Skaftafellsjökull and Kvíarjökull. Table 2.1 lists the different methodologies applied to the samples. Each technique will be thoroughly discussed in the experimental chapter where the technique is used.

### 2.1 Ice sampling

Three sampling campaigns were undertaken. The first sampling campaign took place in June 2012 (by Simon Cook, then at Manchester Metropolitan University, and Darrel Swift, University of Sheffield), when samples of the young and old proglacial soil, subglacial sediment, and supraglacial sediment were collected. For collection, a flame-sterilised ice axe was used to push the sample into 30 ml sterile tubes. For the retrieval of basal ice samples, another two sampling campaigns were required. The first campaign took place in April 2015, and the second one was in May 2016. The sampling protocol that follows was presented in Toubes-Rodrigo et al. (2016).

Prior to the sampling campaigns, satellite imagery was consulted in order to identify potential spots where basal ice could be present. Once on site, the glacier terminus was surveyed to find accessible basal ice sampling locations (Figure 2.1). Much of the glacier and basal ice exposures were covered with surface sediment, and so sluicing was necessary to reveal the ice. Once identified, samples were described, photographed, and coordinates were logged. In order to extract the samples, an ice axe was used to first remove the top 20-30 cm of ice to remove any possible surface cross-contamination. Then, to avoid extra contamination, gloves were worn and an ethanol-flamed chisel was used to remove ice blocks carefully into inside-out sterile bags, avoiding any contact between gloves and ice. Samples were double- or triple-bagged to reduce the risk of contamination due to bag piercing. After sampling, samples were kept frozen at -20°C. Then, upon use, samples were allowed to melt at 4°C in order to limit changes in the chemistry and microbiology.





**Figure 2.1.** Map of all the sampling campaigns performed in Svínafellsjökull glacier. Colours indicate the sampling campaign and the fill in the circles indicate the type of sample

## 2.1 List of methods applied to the samples

**Table 2.1. List of samples and methodologies utilised in this thesis.** Year refers to the year of sampling. XRF – X-ray fluorescence, LECO – Carbon and Nitrogen analysis by LECO TruSpec™, ICP-OES - inductively coupled plasma optical emission spectrometry, IC – ion chromatography, SPA – single particle analysis, NGS – next generation sequencing.

						Chemistry					Cell counts		NGS	Cultivation	
						XRF	LECO	ICP-OES	IC	SPA	Direct	Fluorimetry	Traditional		Ichip
						3	3	3	3	3	4, 5	4	4	5,6	6
Sample	Type	Glacier	Lat	Long	Year										
SUP	Supraglacial sediment	Svínafellsjökull	63.999192	-16.8729	2012										
ST	Subglacial sediment	Svínafellsjökull	63.998878	-16.8775	2012										
MOT	Subglacial sediment	Svínafellsjökull	63.99187	-16.8724	2012										
SPM	Young soil	Svínafellsjökull	63.99928	-16.8776	2012										
IPM	Young soil	Svínafellsjökull	63.99928	-16.8776	2012										
LIA	Old soil	Svínafellsjökull	63.99363	-16.875	2012										
STOR	Old soil	Svínafellsjökull	63.99168	-16.8926	2012										
B1-15	Debris band	Svínafellsjökull	63.99652	-16.878	2015										
B2-15	Debris band	Svínafellsjökull	63.99652	-16.878	2015										
B3-15	Debris band	Svínafellsjökull	63.99652	-16.878	2015										
B4-15	Debris band	Svínafellsjökull	63.99428	-16.8733	2015										
D1-15	Dispersed	Svínafellsjökull	63.99515	-16.8733	2015										

Sample	Type	Glacier	Lat	Long	Year	XRF	LECO	ICP-OES	IC	SPA	Direct	Fluorimetry		Traditional	Ichip
D2-15	Dispersed	Svínafellsjökull	63.99103	-16.8686	2015										
D3-15	Dispersed	Svínafellsjökull	63.99428	-16.8733	2015										
D4-15	Dispersed	Svínafellsjökull	63.99331	-16.8748	2015										
D5-15	Dispersed	Svínafellsjökull	63.99423	-16.8746	2015										
D6-15	Dispersed	Svínafellsjökull	63.99331	-16.8748	2015										
S1-15	Stratified	Svínafellsjökull	63.99428	-16.8733	2015										
S2-15	Stratified	Svínafellsjökull	63.99331	-16.8748	2015										
S3-15	Stratified	Svínafellsjökull	63.99423	-16.8746	2015										
B1-16	Debris band	Svínafellsjökull	63.99877	-16.8764	2016										
B2-16	Debris band	Svínafellsjökull	63.99877	-16.8764	2016										
B3-16	Debris band	Svínafellsjökull	63.99151	-16.8724	2016										
B4-16	Debris band	Svínafellsjökull	63.99151	-16.8724	2016										
B5-16	Debris band	Kvíarjökull	63.94306	-17.0003	2016										
B6-16	Debris band	Kvíarjökull	63.94297	-17.0014	2016										
D1-16	Dispersed	Svínafellsjökull	63.99151	-16.8724	2016										
D2-16	Dispersed	Svínafellsjökull	63.99877	-16.8764	2016										

Sample	Type	Glacier	Lat	Long	Year	XRF	LECO	ICP-OES	IC	SPA	Direct	Fluorimetry		Traditional	Ichip
D3-16	Dispersed	Svínafellsjökull	63.99877	-16.8764	2016										
D4-16	Dispersed	Svínafellsjökull	63.99151	-16.8724	2016										
D5-16	Dispersed	Svínafellsjökull	63.99151	-16.8724	2016										
D6-16	Dispersed	Svínafellsjökull	63.99151	-16.8724	2016										
D7-16	Dispersed	Skaftafellsjökull	64.02794	-16.9333	2016										
D8-16	Dispersed	Skaftafellsjökull	64.02811	-16.9332	2016										
S1-16	Stratified	Svínafellsjökull	63.99151	-16.8724	2016										
S2-16	Stratified	Svínafellsjökull	63.99151	-16.8724	2016										
S3-16	Stratified	Svínafellsjökull	63.9948	-16.8735	2016										
S4-16	Stratified	Skaftafellsjökull	64.02794	-16.9333	2016										
SCDB	Supercool band	Skaftafellsjökull	64.02811	-16.9332	2016										

Samples from 2012 were collected by Simon Cook and were cultured by Robin Sen prior the arrival of the PhD student. The excess of the samples was disposed by one of the members of the technical support, reason for which no chemical analysis could be performed on the aforementioned samples.

James Parker, on behalf of Olympus, lent a Delta Professional Handheld XRF analyser for the 2015 campaign, reason for which only samples from this sampling campaign were analysed using that technique.

Fluorimetry analyses were only performed on the 2016 campaign due to the availability of the instrument, purchased by Prof Mark Enright at the end of 2015.

DNA analysis was prioritised over chemical analysis, and when the amount of sediment recovered was low (especially in dispersed facies, due to the nature of this kind of ice), resources were put towards microbiological analysis instead of geochemical analysis

## 2.2 Methodology caveats

This PhD project has been completed with very low funding, mainly obtained by grant applications. Under these limitations, the first critical decision to make was either increase the replication (having at least three replicates per sample) or increase the representativity, covering a larger extent of the glacier front, and having samples from at least two campaigns to analyse yearly variations. The latter option was chosen over the first one due to the novelty of the project: some studies have assessed the microbial diversity of basal ice, but to our knowledge this project is the first one that tries to identify potential differences in the microbial community of different basal ice facies.

Very extensive chemical analysis was performed in this thesis, both bulk analysis (ICP-OES, IC, XRF), but also micro analysis (SEM-EDX). Mader *et al.*, (2006) designed an equation to calculate the solute concentration in ice veins in clear ice, and showed that this effective concentration was several orders of magnitude higher than the results obtained in the bulk analysis. The main weakness this equation has is that it only accounts for solutes entrapped in veins, but fails to incorporate liquid water around grains. This reason makes the equations easily applicable to clear ice, but fails

to considerate the physical nature of basal ice. One of the assumptions of this thesis was the pH inside of these veins and surrounding the grains was low, but not pH was measured. In previous studies chemical composition ice veins was calculated using Raman spectroscopy (Barletta et al., 2012). For this analysis, it would be ideal that samples are analysed as fresh as possible, especially because Mader *et al.*, (2006) showed that temperature has a very large impact on the ice vein size, and therefore, and impacts greatly on the effective concentration of the different solutes. Samples were sent from Iceland to the UK surrounded by cold blocks, but that would not be enough guarantee that vein size would not change. In addition, what makes basal ice different from englacial ice is the conditions in which it forms beneath glaciers, and refreezing the melted ice in the laboratory would not recreate the same conditions, thus rendering unreliable results.

The mineralogical classification, inspired by Kandler et al., (2009) is very preliminary and some of the categories need to be refined. The large amount of “carbonaceous” minerals might be an artefact, regarding that were loaded onto carbon stickers, which then can appear in the results, especially in particularly small samples, due to interaction volume effects. To correct these artefacts, complementary techniques, such X-ray diffraction (XRD), and Raman spectroscopy can be applied to resolve the mineralogy. Nevertheless, these techniques also have weaknesses, not all minerals show diffraction patterns, and not all minerals show Raman spectra.

This thesis has been the first approach to the microbiology of basal ice in Svínafellsjökull, trying to elucidate if physically-defined facies harbour different microbiota, but also trying to identify the potential metabolic network in this environment. The use of phylogenetic markers, such as ITS and 16S rDNA just offer information of the microbial diversity, although these sequences have been used as a proxy for functionality. Specific software has been developed in order to predict the relationships between the members of the microbial community based on these markers, such as tax4fun (Aßhauer et al., 2015), or PICRUSt (Langille et al., 2013), and it is one of the modules that form part of Parallel-META 3 (Jing et al., 2017). Nevertheless, these results should be regarded as a working hypothesis, and further research should be performed. RNA sequencing (RNA-seq) is a technique that reflects

effective microbial activity, regarding the low stability of RNA. Also, and as mentioned previously in this discussion, DNA can remain in sediment for long periods of time, and persist as relic DNA (Carini et al., 2016), although there are no estimates of how long it would persist in ice. In this PhD project, no means were used to separate active DNA from relic DNA, so it is likely that part of the DNA sequences identified are not part of cells, but appears in the ice as free DNA.

Functional and metabolic network of an environment can be assessed by metagenomics. For this, the whole DNA from the samples is extracted and analysed by shotgun sequencing (e. g. (Venter et al., 2004; Urich et al., 2008; Bohmann et al., 2014)), allowing to not only sequence the 16S rDNA or ITS, but also protein coding-DNA. Again, some of the DNA would be relic DNA, so results would need to be analysed bearing that in mind.

One of the premises of this thesis is that cells are being exported from Svínafellsjökull, and quantification of cells export was performed. Viable cells would be exported from the glacier to the foreland, as the cultivation techniques have proved. Nevertheless, this methodology does not offer a quantitative insight of the real number of cells that are alive in the basal ecosystem, regarding that only a small portion of the microbiota is cultivable (Pham and Kim, 2012). For the assessment of the alive microbiota another set of techniques would be needed, such as LIVE/DEAD assay (e.g. (Blankinship et al., 2014; Jäger et al., 2018)). In addition, direct count is a valid technique, but in case there are any sediment particles, part of the cells can be masked under the grains, resulting in an underestimation of the total number of cells. There are another methodologies than can be used, such as flow cytometry (Irvine-Fynn et al., 2012; Irvine-Fynn and Edwards, 2014), but any presence of grains, again would result in accurate results.

In the last experimental chapter of this thesis, microbial cultivation was performed and some ecological conclusions were drawn. Cultivation allows the recovery of a very small fraction of the total microbiota, as previously mentioned, and therefore ecological conclusions based on cultivation techniques must be regarded as partial. In order to have a wider and more accurate perspective of the microbial diversity different media could have been made, apart from 1:10 TSA and FSM (e. g. Foght et

al., 2004). Different media select for different microbial species, as their nutritional requirements vary. Nevertheless, in an effort to recover a higher microbial diversity, chips were designed and utilised, and this methodology has been hypothesised to recover a significant higher diversity (Nichols et al., 2010). Different species were isolated utilising this method, which has increased the diversity, but because not every single isolated obtained by the two different methods was sequenced, not statistically significant differences between both samples can be calculated.



### 3. Geochemical characterisation of basal ice facies

#### 3.1 Introduction

Hubbard et al. (2009) defined glacier basal ice as, “ice that has acquired a distinctive suite of physical and/or chemical characteristics as a result of processes operating at or near to the bed of an ice mass”. Hence, chemical composition is a key defining property of basal ice. During the 1980s and 1990s, significant advances were made in the chemical characterisation of basal ice (Lorrain & Souchez 1978; Hallet et al., 1978). Compared to englacial ice, basal ice has been shown to be enriched in certain cations, such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , and relatively depleted in  $\text{Na}^+$  and  $\text{K}^+$  (Hubbard et al. 2009) (Table 1.1). Selective enrichment in divalent cations is a consequence of contact between subglacial meltwater and substrate/bedrock such that, after refreezing, these cations become readily incorporated into the basal ice. Monovalent ions are mostly derived from atmospheric precipitation and hence are more abundant in firn-derived englacial ice. Given these different sources, the ratio  $(\text{Na}^+ + \text{K}^+)/(\text{Ca}^{2+} + \text{Mg}^{2+})$  has been used to discriminate between englacial and basal ice (Knight, 1997).

Before the discovery of microbial life in subglacial environments in the mid-1990s (Priscu et al., 1999; Sharp et al., 1999), chemical analysis focussed principally on the use of monovalent and divalent cations to differentiate between basal ice and englacial ice. However, nowadays there is a growing interest in the analysis of basal ice chemical characteristics in order to understand the environment within which microbial life exists (Yde et al., 2010; Mitchell et al., 2013; Montross et al., 2014). For example, Yde et al. (2010) identified carbonates entrapped in basal ice, as well as pyrite and pyroxenes. Tung et al. (2006) also performed identification of minerals entrapped in a basal ice core from the Greenland Ice Sheet, finding an abundance of smectite, and some chlorite. Chemical analysis demonstrated the presence of organic acids in basal ice such as acetate, oxalate, and formate, as well as inorganic ions (nitrate, chloride, sulphate, and ammonia), including an abundance of sulphates. In the same study, organic carbon content was measured; significant concentrations of methane were measured ( $1.2 \times 10^4$  ppmv). Skidmore et al. (2000) found methane content in basal ice at John Evans glacier (Canada) to be  $1.6 \times 10^4$  ppmv, as well as high

concentrations of CO<sub>2</sub> ( $5.8 \times 10^4$  ppmv). Foght et al. (2004) analysed the chemistry of sediment and ice matrix of the basal ice of two glaciers in New Zealand (Franz Josef and Fox) and found low values for C (530, 550 ppm), N (200 ppm), sulphate (180, 145 ppm), phosphate (0.48, 0.15 ppm) for sediment, which were orders of magnitude higher than the corresponding values in the melted ice matrix.

Microbial community composition and abundance can vary depending on the chemical conditions and redox status of the environment that they inhabit, and this is true of basal ice (Mitchell et al., 2013; Nixon et al., 2017). For example, Mitchell et al. (2013) reported a positive correlation between the abundance of Fe- and S-oxidisers and minerals containing these elements in Robertson Glacier (Canada), especially pyrite, which indicated that bedrock mineral composition influenced the bacterial community in the subglacial environment. The presence of certain elements (such as Cu, Zn, Co, Hg, Cr) is detrimental to most microorganisms, meaning that microbial communities become dominated by the minority of species that are adapted to high concentrations of these elements when they are present (Hassen et al., 1998; Silver and Phung, 2005). The growth of microorganisms can be enhanced by the presence of certain elements, which can be used by those microorganisms to obtain energy via chemolithotrophy (Fe, S, N, H) (Claassens et al., 2016) or via anaerobic respiration (Fe, S, N) (Lovley and Coates, 2000). Evidence for these metabolic processes has been found in subglacial environments, including basal ice (Boyd et al., 2010, 2014).

The most popular methods of chemical analysis include atomic absorption spectrometry (AAS) and atomic emission spectroscopy (Melquiades and Appoloni, 2004), but the preparation of samples is rather laborious and time-consuming, leading to an increase in use of other techniques, such as energy dispersive X-ray spectrometry (EDXRF) (Melquiades and Appoloni, 2004). In addition, XRF machinery has been developed to the point that the components have been reduced to in-field portable devices (FPXRF). Use of XRF carries the advantages that it is non-destructive and multi-elemental, and can be applied to any kind of solid and liquid samples, and with high sensitivity (Melquiades and Appoloni, 2004). XRF technologies have been

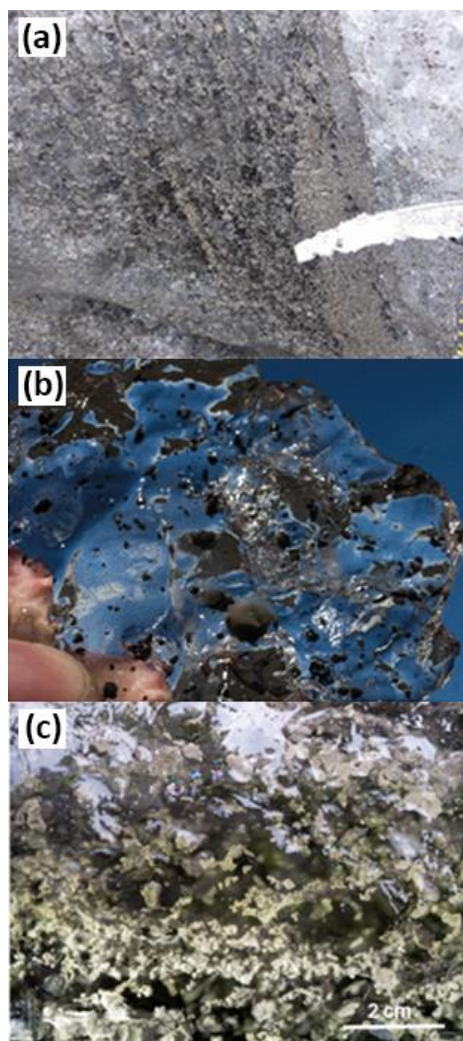
applied in ice samples in order to analyse impurities (Arena et al., 1995; Marcelli et al., 2012)

In addition, XRF has been used previously for the identification of sediment origin, such as in river and shelf sediment in the Yellow sea (Lim et al., 2006), and in sedimentary deposits in Australia (Forbes and Bestland, 2007). However, XRF analysis does not appear to have been applied in studies of basal ice chemistry, which could have helped to clarify the origin of, and differentiate between, different ice facies.

Scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy detection (SEM/EDX) has been used routinely in order to identify individual environmental particles (Worobiec et al., 2010). Automation of this technique has permitted the processing of large numbers of particles (Worobiec et al., 2010). This technique, in combination with bulk analysis, such as XRF or Induced Coupled Plasma – Optical Emission Spectroscopy (ICP-OES), provides very valuable information about the chemical nature of the analysed samples (Worobiec et al., 2010). This is especially relevant for microbiology, since even small distances between points can mean enormous changes in the micro-environment (or micro-niches), and therefore substantial changes in the microbial community (Jones and Bennett, 2014). Techniques such as SEM/EDX provide fundamental information in microbiology since it allows the observation of microorganisms on mineral grains whilst analysing the chemical nature of the mentioned mineral/metals surfaces. In addition, and in combination with Raman, this SEM/EDX allows an even stronger discrimination between minerals based on their spectra (Worobiec et al., 2010), and can even be used to discriminate between bacterial groups (Cardell and Guerra, 2016). Analysis of minerals entrapped in basal ice using EDX has been previously performed (Tung et al., 2006; Yde et al., 2010), but as far as the author is aware this is the first study that has used the EDX coupled with a SEM approach.

Using different methodologies, an extensive characterisation of the chemical nature of basal ice and its components (ice matrix and sediment) at Svínafellsjökull was performed. The aim of the studies described in this chapter are to (1) characterise the chemical nature of the basal ice layer at Svínafellsjökull; (2) assess the extent to

which descriptively different ice facies were chemically similar of different; and (3) Identify the abiotic factors that may influence life in the basal ice ecosystem.



**Figure 3.1. Examples of basal ice facies collected from Svínafellsjökull:** a) debris band (B), composed of sub-vertically layered alternations between clear, bubble-free ice and polymodal sediment – note also the white and bubble-rich englacial ice to the right; b), dispersed facies (D) comprising sparsely dispersed aggregates of polymodal sediment; c), stratified facies (S), rich in fine (clay/silt) sediment, with sediment arranged in angular aggregates; at a centimetre to decimetre scale, stratified facies appears layered, as the name suggests.

## 3.2 Material and methods

### 3.2.1 X-Ray Fluorescence (XRF) analysis

Thirteen samples of accessible basal ice were extracted in April 2015 from Svínafellsjökull (Chapter 2). For *in situ* X-Ray Fluorescence (XRF) analysis of basal ice, the first 20-30 cm of ice was removed in order to avoid any possible surface contamination of the samples, as outlined in (Toubes-Rodrigo et al., 2016). Basal ice is highly heterogeneous in nature (Fig 3.1). Consequently, at least 3 measurements per sample were performed *in situ* by targeting representative parts of the ice (e.g.

clear ice matrix, entrained sediment) using a portable DELTA Professional XRF (Olympus Corp. USA) instrument (Fig 3.2), kindly lent by James Parker on behalf of Olympus for this campaign. The instrument was regularly calibrated using the supplied calibration disc under ambient conditions in the field. For data acquisition, the “GeoChem” mode was used, using 30 seconds per beam, from a 40kV Rh Anode X-Ray tube using a large 30mm<sup>2</sup> Silicon Drift Detector. The two beams used were: Beam 1 - 40kV - 76.7uA - 2mm Aluminium Filter and Beam 2 - 10kV - 52.4uA - No Filter.



**Figure 3.2. *In situ* chemical analysis of a debris band using the DELTA professional XRF analyser.** The effects of the removal of the 20-30 first cm of the basal ice sample can be observed in the picture as an indentation in the ice structure around the analyser.

### 3.2.2 Treatment of extracted ice samples

In order to minimise the risk of changes to the chemistry of extracted ice samples through microbial activity, samples were allowed to melt at  $\sim 4^{\circ}\text{C}$  for a period of up to two weeks. Sediment and liquid fractions were separated by decantation. Liquid samples were filtered through  $0.20\ \mu\text{m}$  Cole-Parmer PTFE Sterile Syringe Filters, into 50 ml Falcon tubes to remove microbial contamination that could modify the chemical composition of the water

### 3.2.3 Sediment concentration analysis

Sediment concentration in the basal ice was calculated in order to quantify any differences between ice facies, and to assess how much of the chemical variability could be explained by the ice matrix versus the sediment. Sediment and water fractions were separated by carefully decanting the liquid fraction into another bag. Both fractions were weighed and then the water fraction was filtered through pre-weighed Whatman Grade 4 cellulose filters. Filters were oven-dried overnight at  $60^{\circ}\text{C}$ . To calculate concentration in the water fraction, samples were filtered through pre-weighed filters. Filters were then dried at  $60^{\circ}\text{C}$  and the difference in the weight corresponded to the sediment weight in the water fraction. For the sediment fraction, samples were transferred into weighted plastic containers and then allowed to dry in an oven at  $60^{\circ}\text{C}$  overnight. The water content was calculated on differential wet/dry weight. Total weight was calculated using these data.

### 3.2.4 Inductively coupled plasma optical emission spectroscopy (ICP-OES)

Samples were vigorously shaken and mixed thoroughly in order to increase the homogeneity and representativeness. Up to 0.5 grams of sediment from each basal ice sample was weighed and transferred to 50 ml PTFE tubes for acid digestion. Depending on the sediment weight, either 25 or 50 ml of reverse aqua regia ( $\text{HCl}:\text{HNO}_3$  1:3) was added to the samples for total metal extraction. Samples were microwaved in a Mars Xpress microwave, which has a maximum power of 1200W (CEM, UK). A 2-step cycle was used. For step 1, 90% of the total power was used with a ramp time of 10 mins, and to a temperature of  $90^{\circ}\text{C}$ , with a hold time of 5 mins. The second step used 100% of the power, with a ramp time of 10 min to a temperature of  $170^{\circ}\text{C}$  and a hold time of 10 min. Microwaved samples were then

filtered using cellulose filters (Grade 540, Whatman, UK) into 50 ml volumetric flasks. Water was added until reaching a volume of 50 ml.

Al, Ca, Fe, K, Mg, Mn, Na, P, and S were analysed using an iCAP 6000 series ICP-OES (Thermo Scientific, UK). For calibration, standards containing all the elements at different concentrations were prepared. The concentration ranges of each analyte, the wavelength at which it was analysed, and the calibration samples are summarised in Table 2.

**Table 3.1. Elements analysed using Induced Couple Plasma – Optical emission spectroscopy.** Table shows the element analysed, the wavelength used, and calibration standard ranges.

Element	Wavelength (nm)	Range (ppm)
Al	394.4	10-200
Ca	422.6	10-100
Cu	324.7	0.5-10
Fe	240.4	10-500
K	76 6.4	10-100
Mg	279	5-50
Mn	257.6	5-50
Na	589.5	10-100
P	178.2	0.5-50
S	180.7	0.5-50
Zn	206.2	0.5-10

### 3.2.5 Carbon and nitrogen content

Exactly 0.2 grams of oven-dried basal ice sediment sample was subjected to dry combustion elemental analysis at 950°C in a Leco TruSpec™ instrument (Thermo Scientific, UK) for total carbon and nitrogen.

### 3.2.6 Ion chromatography

An analysis of the available cations and anions in the ice matrix was performed on the melted filtrate. For this, samples were acidified using reverse aqua regia (3% v/v) in order to mobilise the ions. Two chromatographic columns were used: for cations, an IonPac CG16 guard column and CS16 separation column, using 39mM Methane Sulphonic Acid (MSA) as an eluent; for anions: IonPac AG18 guard column and AS18 separation column, with a Potassium Hydroxide (KOH) eluent from 18mM – 50mM over 16 minutes



### 3.2.7 Sediment Single Particle Analysis (SPA)

Dried basal ice-derived sediment (25 mg) aliquots were suspended in pure methanol and subjected to ultra-sonication using an S-Series Table Top ultrasonicator (Sonicor Inc. USA) in order to obtain a dispersed sample. Fifty  $\mu\text{l}$  of this sediment-laden liquid were then transferred using a pipette to Leit adhesive Carbon Tabs 12 mm (Agar Scientific, UK) mounted on aluminium SEM Stubs 12 mm dia. 6 mm pin (Agar Scientific, UK) for scanning electron microscopy coupled with energy dispersive X-ray spectrometry (SEM-EDX) in a Supra 40VP microscope controlled by SmartSEM software (Carl Zeiss Ltd, UK). For SEM, the acceleration voltage of 25kV was applied and samples viewed at a magnification of 2500X. For *in situ* elemental analysis, the SEM is equipped with a Backscattered Electron Detector (Apollo 40 SDD. EDAX Inc. USA) controlled by Genesis software; optimisation of the scanning time was performed and the best results were obtained using a scanning time of 15 seconds per particle.

### 3.2.8 Data analysis

Averages of the elemental composition of the basal ice samples from the different analytical techniques were calculated, and results were displayed in boxplots using the ggplot2 package (Wickham, 2009) in R studio (R version 3.3.2) (Team, 2013), and potential basal-ice specific elemental correlations assessed via principal component analysis (PCA) using PCA3d package in Rstudio. Light elements (such as H, O, C, N) cannot be measured using XRF, so in order to standardise the results, measured elements were expressed as a ratio between each element and the sum of all measured elements. Confidence ellipses ( $p=0.05$ ) were drawn in the PCA in order to investigate similarities and differences between samples and ice types.

In order to analyse the differences in the concentration of the different elements and ions within the basal ice faces, data sets were evaluated using Kruskal-Wallis analysis. Samples were considered to be significantly different when the p-value was  $< 0.05$ .

For the mineralogical analysis, a script in R (Appendix 1) was developed that transformed the elemental concentration into molar concentration as assigned to mineralogical groups following a modified classification of Kandler et al. (2009). The same script calculated the abundance of minerals and expressed it as percentages



and plotted results as stack bars in ggplots (Wickham, 2009), and different ice facies were compared to identify potential differences in the mineralogy entrapped in basal ice. For this, Kruskal-Wallis testing was used, and a p-value threshold of < 0.05 was used to determine whether samples were significantly different.

In order to assess the reliability of the techniques used in this work, accuracy and precision when possible were calculated. Precision was calculated as the ratio between the standard deviation and the average value per chemical species ( $Precision = \frac{St\ Dev}{Average} \times 100$ ). The accuracy was defined as the deviation in the results compared to an internal control, which composition is known:  $Accuracy = \frac{Average - Control\ concentration}{Control\ concentration} \times 100$ .

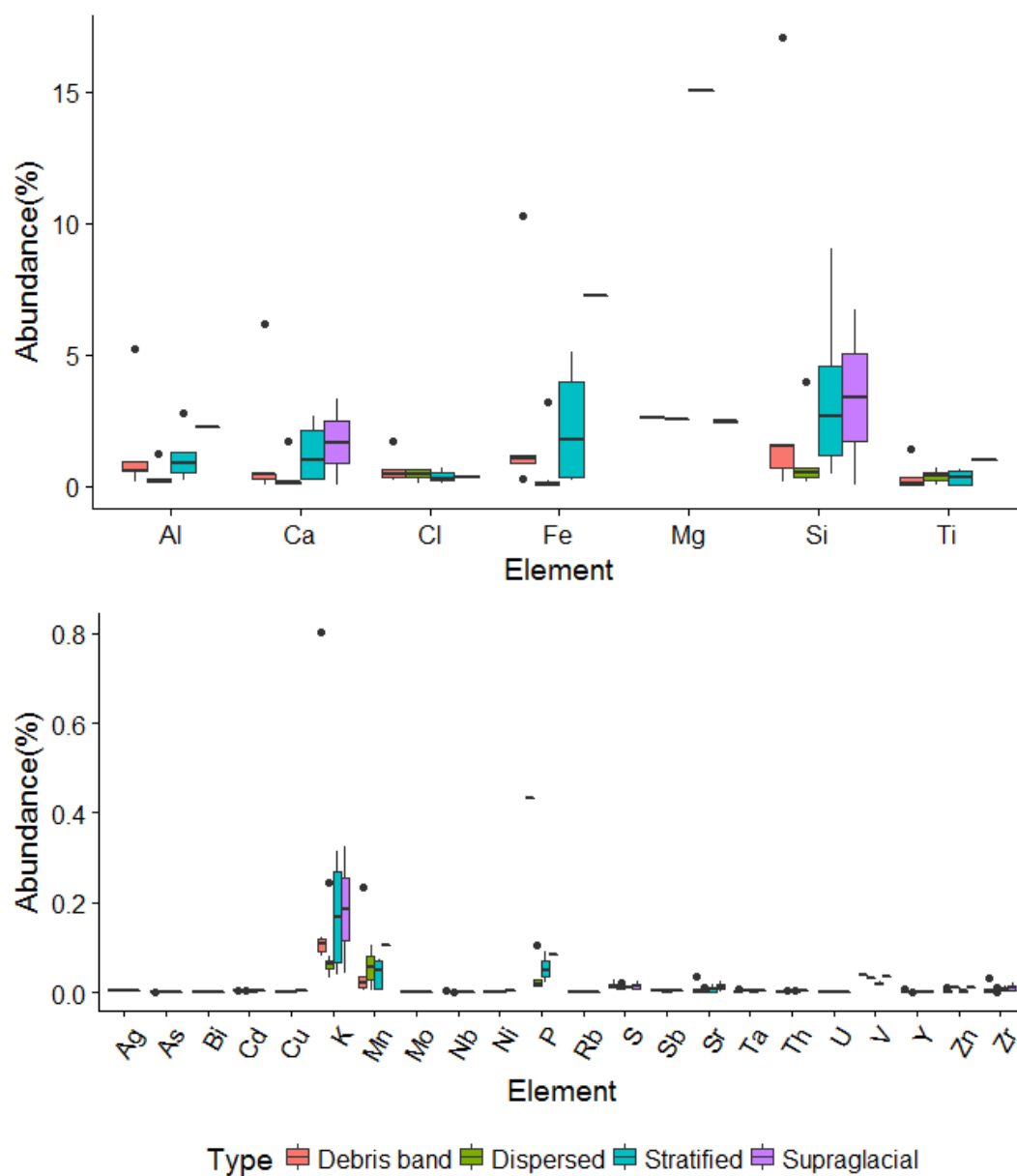
### 3.3 Results

#### 3.3.1 *In situ* XRF of basal ice

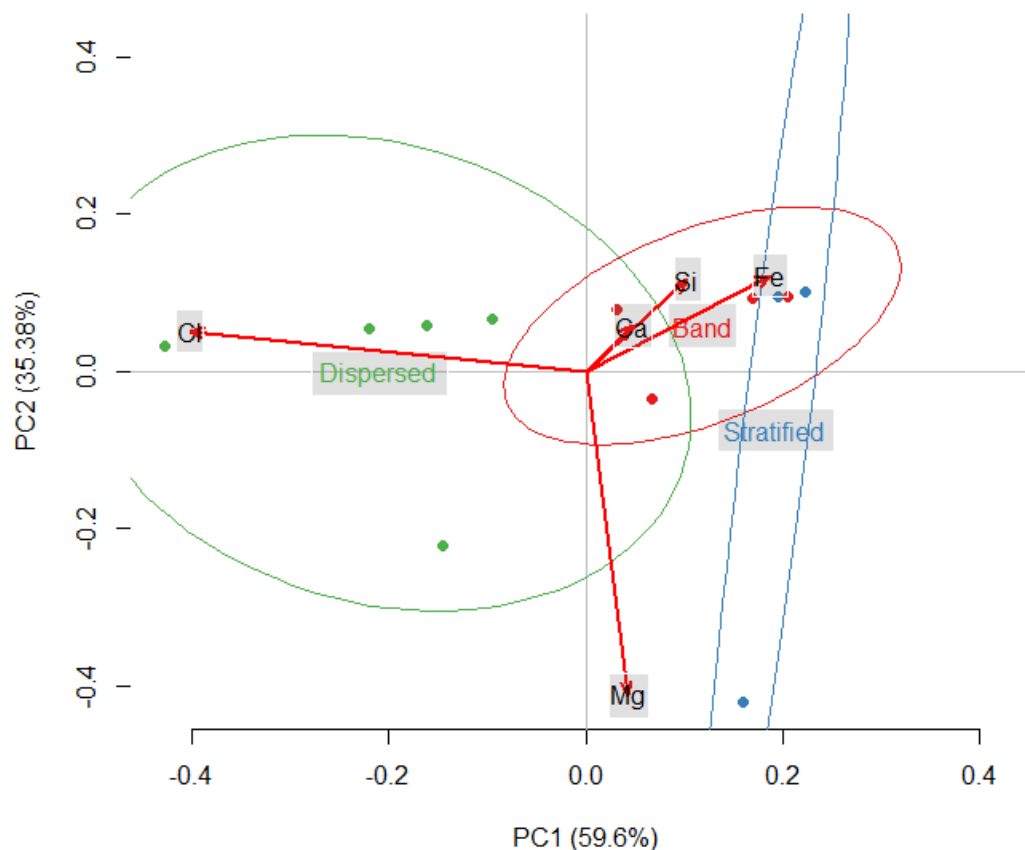
The most abundant elements in all three basal ice facies were Si, Fe, Ca, Mg, and Cl (Figs. 3.3). Two elements showed statistical significant difference between ice types: Fe (p-value=0.01) and Al (p-value=0.01). Mg was particularly abundant in samples from the supraglacial environment. A multivariate analysis (PCA) of basal ice elemental composition (Fig. 3.4) illustrates that stratified and dispersed facies form two distinct sample populations, whilst debris bands possess a chemical composition intermediate between these two end members. The vectored bi-plot analysis highlights greater elemental Cl in dispersed ice as the main driver of difference from other ice types. On the other hand, stratified ice is richer in Fe and Mg, and also Si, Mg and Al.

**Table 3.2. Correlation analysis of the most abundant elements in basal ice.** Linear correlation of the most relevant elements was performed and the table shows the sample pairings where R<sup>2</sup> values were over 0.95

Element pair	R <sup>2</sup>
Al-Si	0.990
Ti-Fe	0.983
Fe-Zn	0.980
Ca-Ti	0.963
Ca-Fe	0.961
Si-K	0.952
Si-Ca	0.951



**Figure 3.3. Box plot of the elemental composition of basal ice analysed *in situ* by portable X-ray fluorescence (XRF).** Red box represents debris bands, green boxes, dispersed, blue, stratified, and purple supraglacial sediment. Lines represent the median and the upper and lower limit of the box represent the 75th and 25th percentile respectively. Dots represent the outliers. The upper graph represents the most abundant elements, and the bottom graph shows rare elements.



**Figure 3.4. Principal Component Analysis of the elemental content of basal ice samples analysed *in situ* by portable X-Ray Fluorescence (XRF) analyser in the 2015 campaign.** Red circles represent debris band samples, green circles represent dispersed facies samples, and blue circles represent stratified facies samples. Confidence ellipses were drawn fixing a confidence value of 95%. Red vectors represent the biplots of the most relevant elements, leading the difference between samples. Values between brackets represent the percentage of variance explained by the axis.

Analysis of the correlation between the different elements showed positive correlation ( $R^2 \geq 0.95$ ) between some elements, which are shown in Table 3.3.

### 3.3.2 Sediment concentration in basal ice

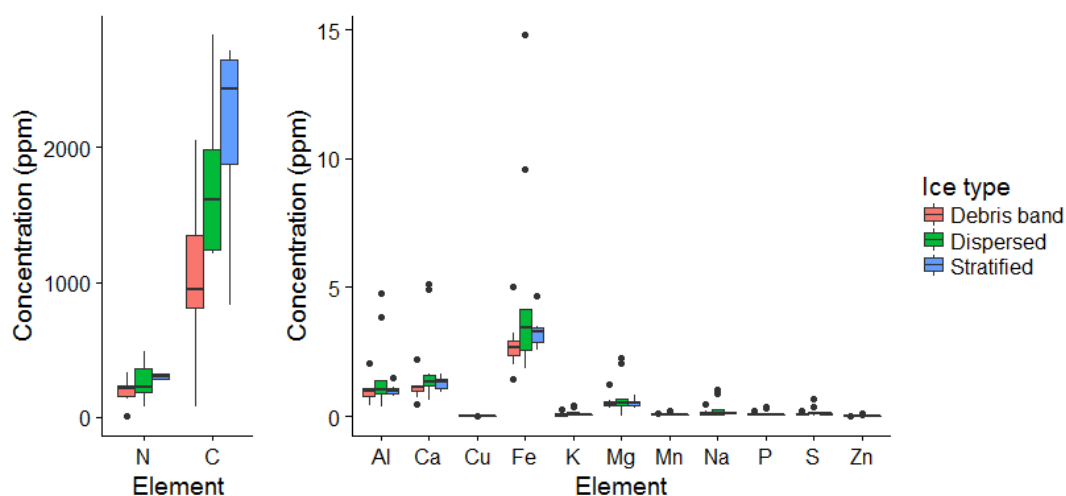
Sediment concentration varied depending on the basal ice type. Concentrations were lowest in dispersed facies at  $1.8 \pm 1.6\%$  ( $n=6$ ). Debris bands had a mean sediment concentration of  $29.7 \pm 24.9\%$  ( $n=6$ ), and for stratified ice sediment concentration was  $45.2 \pm 27.8\%$  ( $n=4$ ). There were statistically significant ( $p < 0.05$ ) differences in sediment concentration between dispersed facies and debris bands and dispersed and stratified facies, but not between debris bands and stratified.

### 3.3.3 Chemical composition of sediment

Sediment entrapped within basal ice was highly depleted in N ( $189.5 \pm 109.3$  ppm for debris bands;  $265.5 \pm 152.2$  ppm for dispersed;  $302.8 \pm 26.9$  ppm for stratified) (Fig. 3.5). Sediment C concentrations were also low ( $1046.5 \pm 668.6$  ppm for debris bands;  $1748.0 \pm 637.2$  ppm for dispersed;  $2102.3 \pm 874.0$  ppm for stratified) (Fig 3.5). No statistically significant differences between ice types for any of the elements analysed were found to exist between sediment samples from different basal ice types.

Elemental analysis (ICP-OES) showed that the most abundant metals in sediment trapped in basal ice were Fe, Ca, Al and Mg (Fig 3.5). For each element, no statistically significant differences were found to exist between different basal ice types.

PCA analysis of elemental composition of sediment also did not show any difference between samples from different basal ice types.

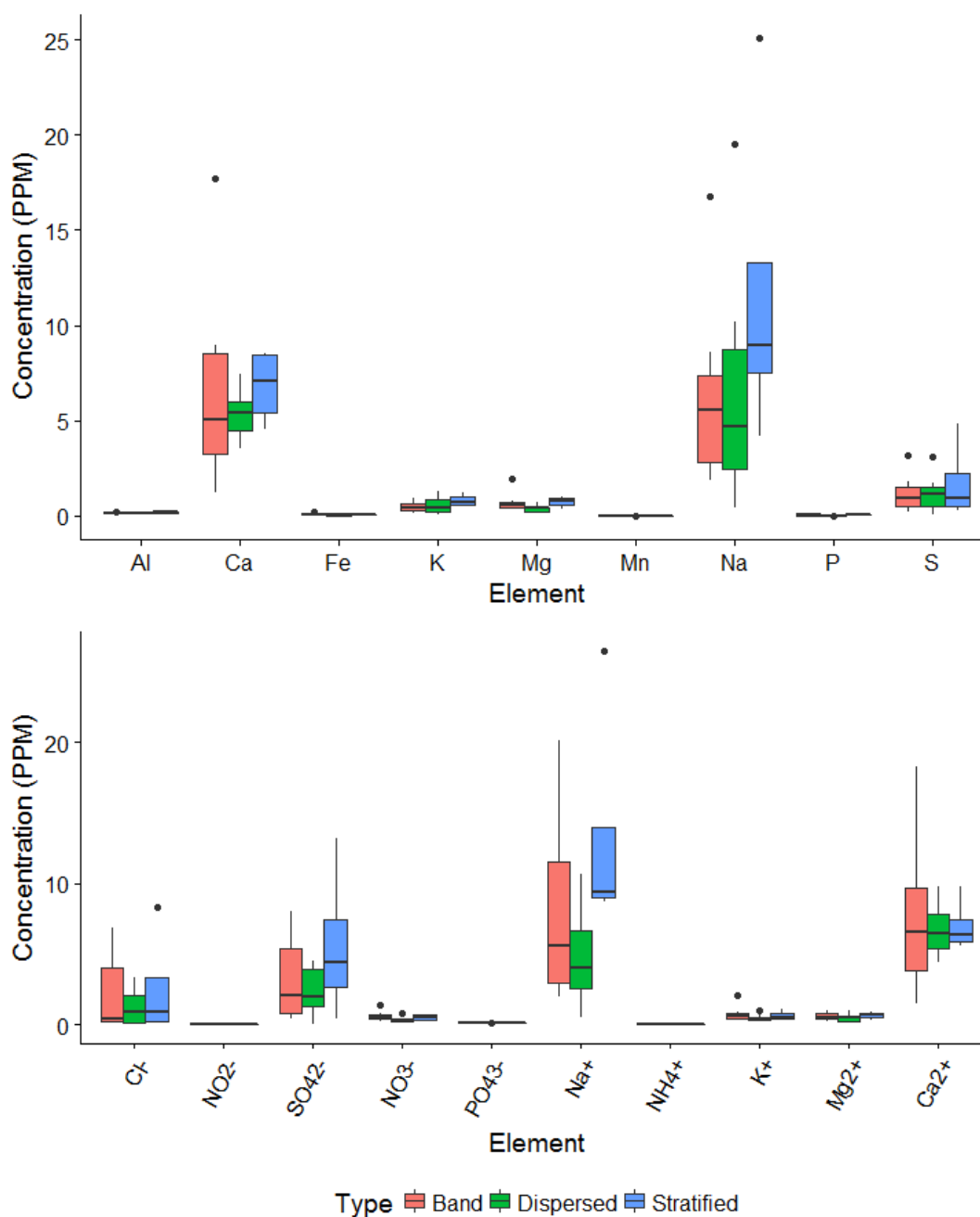


**Figure 3.5. Box plot of the elemental composition of sediment from basal ice. Chemical composition of the basal ice sediment using Combustion Analysis (left) and Inductively Coupled Plasma – Optical Emission Spectroscopy (right).** Lines represent the median and the upper and lower limit of the box represent the 75 and 25 percentiles respectively. Dots represent the outliers. Left graph represents the C and N content of sediment analysed by Leco TrueSpec and right graph represents a multi-element analysis using ICP-OES

### 3.3.4 Chemical composition of the ice matrix

ICP-OES showed the most abundant element in the ice matrix of basal ice were Na, Ca, and S (Fig 3.6). Cl was not analysed by ICP-OES, but appeared to be the fourth most abundant anion by IC (Fig 3.6). Mg and K were also identified by both analyses. Different nitrogen species were analysed by IC, showing the majority of N in the basal

ice matrix appeared as  $\text{NO}_3^-$ , followed by  $\text{NH}_4^+$ . For most samples, the values of  $\text{NO}_2^-$  were below the limit of detection (BLD), and the maximum values recorded were 0.01 ppm. Kruskal-Wallis analysis did not show any significant difference between ice types (p-value < 0.05)



**Figure 3.6. Chemical composition of the basal ice matrix using Inductively Coupled Plasma – Optical Emission Spectroscopy (top) and Ion Chromatography (Bottom).** Lines represent the median, and the upper and lower limit of the boxes represent the 75th and 25th percentile respectively. Dots represent the outliers.

### 3.3.5 Mineralogy of basal ice sediment

A total of 58025 individual particles were analysed by SEM-EDX. All but one of the samples were dominated by silicates, that being B3\_16, which was dominated by unclassified minerals (other) (Fig 3.7). Overall, the dominant minerals were silicates (Debris Bands  $47.3 \pm 17.8\%$ ; Dispersed  $59.9 \pm 13.2\%$ ; Stratified  $51.5 \pm 14.4\%$ ), carbonaceous minerals (Debris Bands  $9.9 \pm 7.6\%$ ; Dispersed  $6.5 \pm 6.8\%$ ; Stratified  $13.0 \pm 9.3\%$ ), Ca-rich silicates (Debris Bands  $6.4 \pm 6.7\%$ ; Dispersed  $4.0 \pm 1.7\%$ ; Stratified  $5.4 \pm 5.5\%$ ), Fe-rich minerals (Debris Bands  $4.7 \pm 6.7\%$ ; Dispersed  $5.8 \pm 2.7\%$ ; Stratified  $4.3 \pm 2.0\%$ ) and carbonaceous quartz (Debris Bands  $3.8 \pm 4.2\%$ ; Dispersed  $5.2 \pm 6.1\%$ ; Stratified  $4.6 \pm 4.2\%$ ) (Fig 3.7). Kruskal-Wallis analysis did not show any differences between abundance of the dominant minerals in the SPA analysis.

### 3.3.6 Error calculations

When possible accuracy and precision values were calculated. For XRF analysis, in order to reduce drift processes, the instrument was recalibrated every three measurements. Values for precision and accuracy were calculated against NIST 2711a standard and are presented in table 3.3. The Olympus Delta analyser has a  $\pm 0.0001\%$  measurement error. As can be observed for the majority of samples both precision and accuracy were high (precision values  $<10\%$ , accuracy values  $>90\%$ ) except P, V, Cd, Ta, Hg, Th, and Ni. In order to analyse how representative the sampling was, average and standard deviations of the measurements on basal ice were calculated and compared by the ratio SD/mean, obtaining that samples in average were highly diverse in average  $70.6\%$  ranging from  $0\%$  to  $245\%$ .

The error for iCAP 6000 series is of  $\pm 0.0001$ . Precision for ICP-OES analysis was calculated, comparing with an internal control. The average precision for the ICP-OES analysis was  $6.2\%$ , ranging  $1.8\%$  to  $8.9\%$ , with an outlier, Na, that was  $36.1\%$ . The average accuracy for the ICP-OES analysis was  $8.6\%$ , but showed abnormalities for K ( $-16.4\%$ ), P ( $170.0\%$ ), S ( $-49.4\%$ ), and Mn ( $-59.2\%$ ).

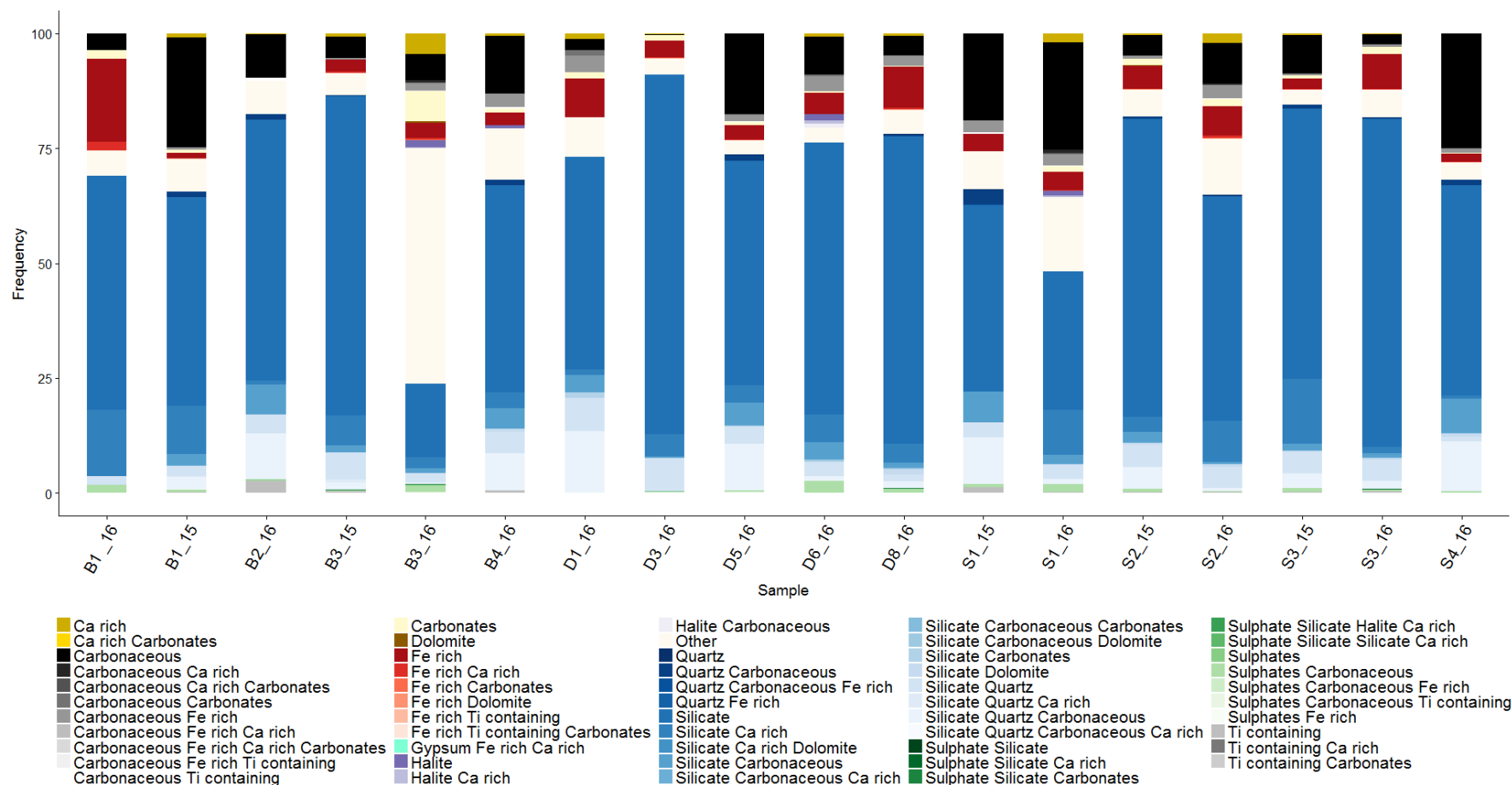
Error for Dionex DX 100–275 is  $\pm 0.0001 \text{ mg L}^{-1}$ . But as no internal errors were run, there is no possibility of calculating accuracy and precision for this assay.

Error for LECO TruSpec is  $\pm 0.0001$  ppm. For LECO analysis accuracy and precision were calculated based on EDTA. Precision values were 0.44% for C and 0.43% for N. Values for accuracy were -0.11% for C and 0.51% for N.

### 3.4 Discussion

#### 3.4.1 Chemical nature of basal ice

Three basal ice facies were identified and sampled at Svínafellsjökull: debris bands, dispersed facies, and stratified facies, which in previous studies have been suggested to have formed through different processes, such as regelation (Johannes Weertman, 1957; Hubbard and Sharp, 1993), glaciohydraulic supercooling (Lawson et al., 1998; Cook et al., 2007, 2010), folding and faulting of sediment and previous basal ice (Goodsell et al., 2002). These processes will confer distinctive characteristics to basal ice: poor in bubbles, rich in sediment, enrichment in heavier isotopes (Knight, 1997; Hubbard et al., 2009), as well as enrichment in solutes derived from the sediment underneath, such as  $Mg^{++}$  and  $Ca^{++}$  (Lorrain and Souchez, 1978; Lorrain, R D; Souchez, R A; Tison, 1981). Results from chemical analysis in the present work showed that Ca was among the most abundant elements analysed by XRF ( $0.8 \pm 0.9\%$  debris band,  $0.12 \pm 0.06\%$  dispersed,  $1.2 \pm 0.5\%$  stratified) (Fig 3.3). It also appeared to be one of the most abundant elements in the sediment (debris band  $1.1 \pm 0.5$  ppm, dispersed  $2.0 \pm 1.6$  ppm, and stratified  $1.3 \pm 0.3$  ppm) (Fig 3.5) and ice matrix (debris band  $6.8 \pm 5.6$  ppm, dispersed  $5.4 \pm 1.3$  ppm, stratified  $6.8 \pm 2$  ppm) (Fig 3.6). Ca was significantly more abundant dissolved in the ice matrix than in the sediment (Kruskal-Wallis p-value =  $1 \times 10^{-6}$ ). In addition, Ca was one of the elements



**Figure 3.7. Mineralogical analysis of the sediment entrapped in basal ice using Single Particle Analysis (SPA) by Scanning Electron Microscopy coupled with Energy Dispersive X-ray (EDX).** B corresponds to debris bands, D corresponds to dispersed facies, and S corresponds to stratified facies. The 2-digit code after the underscore corresponds to the sampling year, 2015 or 2016. Particles were classified following using the R script in Appendix 1, and following a similar protocol to Kandler (2009), based on concentration and ratios between different elements contained in the grains.



that led the differences between ice types in basal ice in the PCA (Fig 3.4). Ca rich minerals appeared between the particles analysed by SPA (Fig 3.7), which drives this separation, regarding the highest concentration of sediment in both debris bands and stratified. Si was the most abundant element in the XRF analysis (debris band  $2.1\pm2.5\%$ ,  $0.4\pm0.2\%$  dispersed,  $1.2\pm0.5\%$  stratified) (Fig 3.3), which agrees with the SPA results that showed that most abundant minerals in almost all samples were silicates (Fig 3.7).

Fe was the third most abundant element in the result from the XRF analysis, and it was particularly abundant in stratified facies ( $2.3\pm1.4\%$ ) and debris band ( $1.3\pm1.5\%$ ) but low in dispersed ( $0.09\pm0.06\%$ ) (Fig 3.3). In the sediment, it was the most abundant element analysed by ICP-OES (debris band  $2.8\pm1.1\text{ppm}$ , dispersed  $4.9\pm4.1\text{ppm}$ , stratified  $3.3\pm0.7\text{ppm}$ ) (Fig 3.5) showing no statistically significant differences between sediment from different ice types, but values were significantly lower (Kruskal-Wallis p-value =  $2.5\times10^{-8}$ ) in the ice matrix (debris band  $0.08\pm0.07\text{ppm}$ ,  $0.04\pm0.02\text{ppm}$ ,  $0.06\pm0.03\text{ppm}$ ) (Fig 3.6). This result would indicate the Fe appears in an insoluble form, mainly as part as the mineral grains. The differences between Fe concentration in the ice observed in the XRF results can be explained by the high Fe-rich sediment content that debris band and stratified show ( $29.7\pm24.9$  and  $45.2\pm27.8\%$  respectively), compared to the low sediment concentration in the dispersed facies ( $1.8\pm1.6\%$ ). Al was the fourth most abundant element in the XRF analysis (debris band  $0.73\pm0.71\%$ , dispersed  $0.16\pm0.09\%$ , stratified  $0.95\pm0.07\%$ ) (Fig 3.3), and showed statistical significant differences (Kruskal-Wallis p-value=0.01) between ice types, being particularly abundant in stratified, followed by debris band. Al was the third most abundant element in the sediments analysed by ICP-OES (debris band  $1.0\pm0.5\text{ppm}$ , dispersed,  $1.6\pm1.5\text{ppm}$ , stratified  $1.0\pm0.2\text{ppm}$ ) (Fig 3.5), but similarly to the case of Fe, values of this element in the ice matrix were significantly lower ( $2.5\times10^{-8}$ ) (debris band  $0.16\pm0.03\text{ppm}$ , dispersed  $0.14\pm0.01\text{ppm}$ , stratified  $0.20\pm0.05\text{ppm}$ ) (Fig 3.6). Surprisingly Cl, which was the main vector leading the separation between dispersed facies and the rest of the ice types did not show any significant differences either in the XRF analysis (p-value=0.09), or in the IC analysis (p-value=0.9) and no chlorides appeared in the SPA analysis (Fig 3.7). Sulphur was

among the most abundant elements in the ice matrix (debris band  $1.2 \pm 1.0$  ppm, dispersed  $1.2 \pm 1.0$  ppm, stratified  $1.7 \pm 2.1$  ppm) analysis (Fig 3.6), but values were significantly lower in the sediment ( $p$ -value =  $1 \times 10^{-6}$ ).

The results indicate that Si, Fe, and Al appear mainly in the sediment, where form minerals such as silicates and Fe-rich minerals (magnetite, goethite, olivine). On the other hand, the ice matrix is richer in the soluble forms of Ca (as  $\text{Ca}^{++}$ ), S (as  $\text{SO}_4^{2-}$ ).

#### 3.4.2 Origin and relationship between the basal ice facies

Using chemical analytical techniques differences have been identified between these ice facies that can assist in their classification and in understanding their origins (e.g. Fig 3.4). Glaciers are known to comprise morphologically distinct basal ice facies exhibiting differing physical-chemical features that supports the view that basal ice is produced by a variety of processes operating near the ice-bed interface (Hubbard et al., 2009). A series of previous studies of basal ice formation at Svínafellsjökull have argued that:

- 1) Stratified facies basal ice forms either through regelation, or through glaciohydraulic supercooling (Cook et al., 2007, 2010). The basal ice sampled in this study is descriptively consistent with facies attributed previously to supercooling. Glaciohydraulic supercooling occurs when the melting point of ice is depressed by thick ice overburden, allowing the water to remain liquid below  $0^\circ\text{C}$ . This is particularly effective in subglacial overdeepenings, which provide thick ice that thins toward the margin, steep reverse slope, and flat ice surface profile (Cook et al., 2006). Water is forced out along a pressure gradient along the adverse slope. As it ascends, the pressure decreases and the melting point rises towards  $0^\circ\text{C}$ . For the water to remain in thermal equilibrium with the overlying ice, the water must warm as it moves along the gradient of decreasing pressure. Heat can be provided by viscous dissipation, geothermal heat flux, basal sliding, and/or latent heat released as the water freezes. (Alley et al., 1998; Lawson et al., 1998; Cook et al., 2006).
- 2) No previous studies have examined debris band formation at Svínafellsjökull directly, but Cook et al. (2011b) argued that the dispersed facies formed through the evolution of debris bands originally entrained at the ice fall. The

origin of debris bands has proven to be controversial (e.g. Moore et al., 2010, 2011), but broadly, they are commonly composed of basal sediment and ice that is consistent with a basal freezing or regelation origin (Hambrey et al., 1999; Hubbard et al., 2004). At Svínafellsjökull, the conditions for englacial sediment entrainment and debris band formation may be met beneath the icefall where there is the potential for intense compression and the folding and thrusting of basal ice and sediment into englacial, and even supraglacial, locations (cf. Goodsell et al. 2002; Swift et al., 2006). The surface expression of these features could be represented by band ogives visible on the glacier surface beneath the icefall. Flow against the terminal overdeepening may lead to reactivation of debris bands through longitudinal compression (Darrel Swift et al., 2006; Cook et al., 2011b). Dispersed facies were hypothesised by Cook et al. (2011b) to evolve from debris bands. Specifically, they envisaged that the basal part of band ogives (i.e. sediment-laden thrusts and folds formed at the base of the icefall) would be metamorphosed by strain and regelation to form dispersed facies.

From these previous studies, it can be hypothesised that debris bands and dispersed facies should share chemical characteristics because of their alleged shared origins; stratified facies should appear distinct from dispersed facies because they are sourced from different parts of the glacier bed (stratified from below the terminus, and dispersed from beneath the icefall); debris bands may share similarities with stratified facies if material from the bed is entrained along debris bands or shear planes that were initiated near the ice fall and reactivated close to the terminus by flow compression against the adverse bed slope.

XRF highlighted one of the intrinsic characteristics of basal ice: its high variability ( $SD/average = \sim 70\%$ ). Basal ice shows different components: sediment and ice matrix, and when clearly distinguishable, XRF analysis aiming to these components was performed. Basal ice sediment can be derived from different parts of the bedrock, and have been mixed during mix time during glacier flow overtime. In addition, the ice matrix can also be a mix of firnified-derived englacial ice, and subglacially-formed basal ice. The consequence of this process would lead to a high heterogeneity in the

basal ice, even within very small distances. This information is critical, since bulk analysis, such as ICP-OES, or IC will fail to account for this heterogeneity.

PCA of chemical composition by XRF showed that debris bands shared similarities with both dispersed and stratified facies, but that stratified and dispersed facies are distinct from one another (Fig 3.4), which lends some support to these hypotheses. The differentiating elements are Fe and Mg for stratified, and Si and Ca to a lesser extent; and Cl for dispersed facies. The mineralogy of the sediment entrapped in basal ice can explain these differences. Firstly, both debris bands and stratified facies are richer in sediment ( $29.7 \pm 24.9\%$  for debris bands and  $45.2 \pm 27.8\%$  for stratified, compared to only  $1.8 \pm 1.6\%$  in dispersed), so will have higher amounts of debris-derived elements (Fig 3.7). SPA showed that the most abundant minerals entrapped in basal ice were Si-rich minerals (silicates), which agrees with the *in-situ* elemental (XRF) data of intact facies (Fig 3.3). The XRF analysis showed that Si co-occurred with Al, Ca, and Fe (Table 3.4). Si is associated with Al and Ca in feldspars, such as plagioclase, which was found in high abundance in sediment samples from Svínafellsjökull. Fe appeared as one of the dominant elements both in XRF and ICP-OES analyses of sediment (Fig 3.3 and Fig 3.5), but the values for this element in the ice matrix were very low (Fig. 3.6). Fe can appear as soluble or insoluble Fe, depending on its oxidation state (Fe(II) or Fe(III)), which varies depending upon the pH and redox conditions. Previous work showed the existence of anaerobic pockets in subglacial environments, which would favour the presence of insoluble iron (Skidmore et al., 2000; Wadham et al., 2008). On the other hand, Barletta et al., (2012) showed that brine veins within the ice matrix can be acidic, favouring the solubilisation of Fe. The presence of active microbial cells in the ice veins would deplete the environment of oxygen, which would generate conditions for the Fe to remain in an insoluble form, as appeared in the analysis.

### 3.5 Conclusions

Chemical analysis of the sediment and ice matrix in basal ice showed that there is fractionation from some elements, and whilst some elements were significantly more abundant in the ice matrix (S, Ca), some of them appeared preferably as part of the sediment (Fe, Al).

XRF analysis showed that there were statistical significant differences for some elements depending on the ice type: Fe and Al showed higher abundance in debris bands and stratified facies.

PCA of the chemical composition obtained by XRF of the different basal ice facies identified in Svínafellsjökull showed that dispersed and stratified facies are different, and that debris bands occupy an intermediate position.

XRF has been shown to be a very useful technique in order to identify differences in the basal ice facies identified in Svínafellsjökull, which can help to identify their origin and how they are related.

## 4. Culture-independent characterisation of bacterial and fungal diversity within basal ice at Svínafellsjökull

### 4.1 Introduction

As indicated in Chapter 1 Literature Review, sediment-bearing basal ice forms as a consequence of interaction between ice and the underlying bedrock or sediment, which imparts a set of distinctive chemical and physical characteristics to basal ice (Hubbard et al., 2009). Before the late 1990s, the subglacial environment was assumed to be sterile, particularly with regard to microbes (Yde et al., 2010), but nowadays it is known that the abundance of microbial cells is high, from  $2.5 \times 10^2$  -  $1.2 \times 10^4$  g ml<sup>-1</sup> within basal ice and  $7.9 \times 10^6$  cells g<sup>-1</sup> within basal sediment from Taylor glacier Antarctica (Stibal, Hasan, et al., 2012; Montross et al., 2014),  $8.7 \times 10^5$  cells g<sup>-1</sup> in Greenland (Stibal, Hasan, et al., 2012),  $1.7 - 6.8 \times 10^5$  cells g<sup>-1</sup> in basal ice in Finsterwalderbreen, Svalbard (Lawson et al., 2015). The first studies confirming presence of viable bacteria within the subglacial environment were Sharp et. al (1999) from Alpine glaciers, and Priscu et al. (1999) from basal ice from above a subglacial lake in Antarctica. More recently, there has been an increasing literature on characterising the abundance and diversity of microbes in the subglacial environment, which differs markedly from the better understood supraglacial and englacial environments (Vilmundardóttir et al., 2014). However, most of the microbiological studies that have targeted subglacial environments have focused on subglacial lakes (Mikucki and Priscu, 2007; Marteinson et al., 2013; Mikucki et al., 2016) and subglacial sediment (Foght et al., 2004; Stibal, Hasan, et al., 2012; Mitchell et al., 2013). Few studies to-date have specifically reported on identity and function of microbial communities in basal ice (Skidmore et al., 2000; Yde et al., 2010; Montross et al., 2014), and fewer still have sampled accounting for facies variability within basal ice.

The interaction between ice and bedrock means that the local subglacial geological conditions represent a first-order control on the nature and composition of basal ice and probably, therefore, on the microbial habitat. Sediment entrained within the ice matrix represents significant nutrient and redox energy sources, which makes basal

ice a suitable environment for microbial colonisation (Skidmore et al., 2000; Montross et al., 2014; Nixon et al., 2017). The mineralogy of the sediment entrapped in basal ice will determine the abundance and composition of the microbial community (Mitchell et al., 2013). Furthermore, the presence of mineral grains in the ice matrix guarantees maintenance of liquid water films adhered to mineral grain surfaces (Tung et al., 2006), with further liquid water present within the extensive networks of veins between ice crystal boundaries (Mader et al., 2006). High water solute concentration within veins of an ice matrix results from active solute exclusion during ice crystal formation (Mader et al., 2006).

Liquid water is a prerequisite for active microbial life and, unsurprisingly, viable microbial cell accumulation in liquid water films and veins has been identified in the ice matrix (Tung et al., 2006; Buford Price, 2007; Bakermans and Skidmore, 2011). These studies have shown that microbial enrichment and selection in solutes greatly depends on the nature of the ice: in Antarctica, the brine veins are enriched in sulphuric acid, therefore selecting an acidophilic community, whereas sea ice brine veins are enriched in salt, selecting for halophiles (Mader et al., 2006). The debris and sediment entrapped in the basal ice and resulting solute accumulation in brine liquid veins are potential C and energy sources for supporting an active microbial community in basal ice, as life has been proven to thrive in other dark, mineral-rich environments such as the Earth's subsurface (Stevens, 1997; Amend and Teske, 2005; Momper et al., 2017) or the ocean floor next to hydrothermal vents (Parnell et al., 2002; Tyler et al., 2002)

The absence of visible light in the subglacial environment precludes reliance on energy production from autotrophic C fixation (photosynthesis). Consequently, subglacial carbon fixation powering primary production will rely on chemolithotrophy, where minerals are oxidised in order to produce energy via inorganic carbon fixation used to generate organic matter (Boyd et al., 2011; Mitchell et al., 2013).

Very recent studies have characterised the presence of chemolithotrophic bacteria in the subglacial basal ice in glaciers (e.g. Russell Glacier and Taylor Glacier) (Mitchell et al., 2013; Boyd et al., 2014), such as *Thiobacillus*, *Acidithiobacillus*, or

*Siderooxidans*, which, due to their activity, fix C into organic matter that can then be used by heterotrophic bacteria, fungi, and archaea. Bacterial phylogenetic markers (16S rDNA) linked to heterotrophic bacteria such as *Paenisporosarcina*, *Bacillus*, *Paraliobacillus* (Doyle et al., 2013), *Polaromonas*, *Lysobacter*, *Clostridium* (Stibal, Hasan, et al., 2012; Rime et al., 2016), and fungi (*Cryptococcus*, *Rhodotula*, *Penicillium*) have been found in basal ice (Sonjak et al., 2006; Butinar et al., 2007; D'Elia et al., 2009). There is still very little information about the fungal diversity in basal ice.

The working hypothesis of this chapter is that different ice types, regarding the differences in the chemistry observed in Chapter 3, would harbour different microbial communities. In order to test this hypothesis and address some of the shortcomings in our knowledge of the microbiology of basal ice and its relevance the main aims of the studies reported in this chapter centre on (1) microbial cell enumeration in basal ice; (2) a culture-independent molecular phylogenetic characterisation of bacteria and fungi that inhabit glacier basal ice; and (3) with reference to basal ice geochemistry (Chapter 2), identification of potential functional relationships of these primary producers, which represent two key domains of life.

## 4.2 Materials and Methods

### 4.2.1 Ice sampling

Accessible basal ice facies at Svínafellsjökull were sampled aseptically in April 2015 and May 2016 following (Toubes-Rodrigo et al., 2016). Sampling targeted the three distinct facies - dispersed, stratified and debris band (Cook et al., 2011a, b). The top 20-30 cm of basal ice was first removed using an ice axe to avoid surface contamination. Basal ice was then sampled aseptically using an ethanol-flamed chisel, and the underlying ice fragments collected in sterile double or triple zip lock plastic bags in order to avoid cross-contamination due to ice shard piercing. Individual basal ice samples were allowed to melt at 4°C in the bags (Stibal et al., 2015).

### 4.2.2 Microbial content in basal ice

Ice samples were melted in Falcon tubes and samples for microbial counts were fixed using formaldehyde at a final concentration of 4% v/v before storage at ~4°C until



further analysis. Back in the laboratory, sediment and supernatant were separated; fixed samples were sonicated for 3 minutes using an S-Series Table Top ultrasonicator (Sonicor Inc. USA) at maximum intensity to release the cells from the sediment grains. 1 ml was extracted from the supernatant and filtered using 0.2 µm Whatman® Nuclepore™ Track-Etched Membranes. For cell enumeration of the sediment present in basal ice samples, dried filters were stained using 4'-6-diamidino-2-phenylindole (DAPI) (1 mg ml<sup>-1</sup>) and covered with Leica type P immersion liquid prior to examination and cell enumeration in an epifluorescence microscope Nikon Eclipse E600 (Nikon, Japan) (Lunau et al., 2005).

#### 4.2.3 DNA extraction and sequencing

Total genomic DNA extracted using MoBio kits followed the manufacturer's instructions. For the 2015 samples, the PowerSoil® kit was used to extract DNA on 0.25g (wet weight) samples of basal ice sediment. However, as relatively low DNA yields were achieved, the PowerMax® Soil DNA Isolation kit was employed in the subsequent 2016 campaign allowing DNA extraction from up to 10 g of wet sediment according to the manufacturer's instructions. DNA extracts were maintained at -20°C prior to analysis. DNA concentration was quantified by fluorescence using a FLUOstar Omega plate reader (BMG Labtech, UK) with reference to a calibration curve (0.12 to 0.004 ng/µl) following the manufacturer's instructions.

Landenmark et al., (2015) estimated that C associated with DNA represented 3% of cells' total C. In addition, Whitman et al., (1998) estimated that in oligotrophic environments, such as basal ice (Margesin and Miteva, 2011; Montross et al., 2014), the C content in bacterial cells was 20 fg. Combining both works, the total numbers cells was calculated as:

$$\text{Equation 1. } \text{cells} = \frac{[DNA](\mu\text{g/ml})}{\text{sediment weight (g)}} \times \frac{100 (\text{total cell C})}{3 (\text{DNA-associated C})} \times \frac{1 \text{ cell}}{20 \text{ fg C}} \times 10^9 (\text{fg to } \mu\text{g conversion})$$

Samples were amplified for sequencing in a two-step process. The forward primer was constructed with (5'-3') the Illumina i5 sequencing primer (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG) and the 357F primer (5'-CCTACGGGNGGCWGCAG-3'). The reverse primer was constructed with (5'-3') the Illumina i7 sequencing primer (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG) and

the 785R primer (GACTACHVGGGTATCTAATCC). For fungal ITS amplification, the primers were ITSF1 (CTTGGTCATTTAGAGGAAGTAA) and ITS2aR (GCTGCGTTCTTCATCGATGC). Amplifications were performed in 25 µl reactions with Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), 1 µl of each 5uM primer, and 1 µl of template. Reactions were performed on ABI Veriti thermocyclers (Applied Biosystems, Carlsbad, California) under the following thermal profile:

95°C for 5 min, then 10 cycles of 94°C for 30 secs, 50°C for 40 secs (+0.5°C per cycle), 72°C for 1 min, followed by 25 cycles of 94°C for 30 secs, 54°C for 40 secs, 72°C for 1 min, and finally, one cycle of 72°C for 10 min and 4°C hold.

Amplification products were visualized with eGels (Life Technologies, Grand Island, New York). Products were then pooled equimolar and each pool was size selected in two rounds using Agencourt AMPure XP (BeckmanCoulter, Indianapolis, Indiana) in a 0.75 ratio for both rounds. Size selected pools were then quantified using the Quibit 2.0 fluorometer (Life Technologies) and loaded on an Illumina MiSeq (Illumina, Inc. San Diego, California) 2x300 flow cell at 10pM. The average number of reads per samples was 10K reads.

#### 4.2.4 Bioinformatics

Raw sequence reads (FastQ) were processed using Mothur: sequences were trimmed using the make.contigs command, then aligned (align.seqs), and filtered (filter.seqs). In order to check for quality, a sequence length distribution analysis was performed and sequences that were over and below the median were removed. In order to identify potential chimeric sequences, the function chimera.uchime was used and removed from the trimmed file using remove.seqs. Sequences were aligned and processed using Parallel-META 3.4.1 pipeline (Jing et al., 2017) to analyse the paired end 16S sequences. Parallel-META has been used previously in different studies using bacterial 16S rDNA (i.e. (Escobar-Zepeda et al., 2016; Gao et al., 2017; Wang et al., 2017)). The taxonomic levels analysed were: Phylum and Genera, using the “-L 15” command in Parallel-META. The Phyla, Genera and operational taxonomic unit (OTU) tables generated were then used for further analysis. For the OTU clustering, a threshold of 97% homology was chosen. In order to analyse the sequencing depth, rarefaction curves were drawn using the -R T function in Parallel-

META. The minimum sequence count threshold was 2; abundance thresholds were fixed for maximum 0.1% and minimum 0%, minimum no-zero abundance threshold 10% and minimum average abundance threshold 0.1%. OTU identification was performed using the SILVA database built in Parallel-META 3.4.1 (v123). The outputs of the analysis were OTU distribution table, genera distribution table, phyla distribution table, and a network analysis.

#### 4.2.5 Statistical analysis

Principal Component Analysis (PCA) was performed using R 3.3.2 (Team, 2013) and the package *pca3d* (Weiner, 2014) to visualise the overall community structure and identify differences between the different ice types. The OTUs corresponding to vectors represented in the biplot were identified using BlastN built in Mega7 (Kumar et al., 2016), in order to find the nearest identified organisms and the environment of isolation.

Bacterial and fungal community representation was summarised at phylum and genera abundance levels. Genera above 2.5% abundance were reported with genera below this threshold, designated as a single group termed “Others”. Differences in phyla abundance between ice types at genus level were identified using Kruskal-Wallis analysis, and significant groups ( $p < 0.05$ ) were plotted using the “ggplots” (Wickham, 2009) package in R studio.

### 4.3 Results

#### 4.3.1 Enumeration of microbial cell counts in basal ice

##### **Direct count**

Cell concentrations in sediment samples from 2015, irrespective of ice type, were  $\sim 10^7$  cells  $\text{g}^{-1}$  (Table 4.1) of sediment, without any significant difference between ice types (Kruskal-Wallis analysis  $p > 0.05$ ). Ice matrix samples showed much lower cell concentrations, on the order of  $\sim 10^5$  cells  $\text{ml}^{-1}$  of ice, and again, no significant difference appeared between ice types (Kruskal-Wallis analysis  $p > 0.05$ ). However, there are significant differences between the cells concentration in the sediment ( $x = 1.4 \pm 0.4 \times 10^7$  cells  $\text{g}^{-1}$ ) and the ice matrix ( $x = 2.1 \pm 0.5 \times 10^5$  cells  $\text{ml}^{-1}$ )

**Table 4.1. Direct cells counts using DAPI from samples from the 2015 field campaign at Svínafellsjökull.**

	Sediment	Ice
Sample	Cell g <sup>-1</sup>	Cell ml <sup>-1</sup>
B1	2.0x10 <sup>7</sup>	3.4 x10 <sup>5</sup>
B4	1.3 x10 <sup>7</sup>	1.4 x10 <sup>5</sup>
D1	1.3 x10 <sup>7</sup>	2.5 x10 <sup>5</sup>
D3	8.6 x10 <sup>6</sup>	2.4 x10 <sup>5</sup>
D6	2.2 x10 <sup>7</sup>	2.0 x10 <sup>5</sup>
S1	1.4 x10 <sup>7</sup>	1.5 x10 <sup>5</sup>
S2	1.5 x10 <sup>7</sup>	2.5 x10 <sup>5</sup>
S3	8.9 x10 <sup>6</sup>	1.2 x10 <sup>5</sup>

Cell concentrations calculated on the basis of DNA fluorescence (Table 4.2) showed that debris bands harboured  $9.4 \pm 2.5 \times 10^5$  cells g<sup>-1</sup>, dispersed  $6.9 \pm 4.5 \times 10^5$  cells g<sup>-1</sup>, and stratified  $5.6 \pm 1.4 \times 10^6$  cells g<sup>-1</sup>, 2.5 showing statistically significant differences between stratified and band (p-value =  $3 \times 10^{-7}$ ), and stratified and dispersed (p-value =  $1 \times 10^{-7}$ )

**Table 4.2. DNA concentration in basal ice sediment after extraction using MoBio PowerMax™ based on fluorimetry assay. B (debris band), D (dispersed facies) and S (stratified facies).**

Sample	DNA (µg ml <sup>-1</sup> )	Cells (cells g sediment <sup>-1</sup> )
B1_16	5.0 x10 <sup>-3</sup>	8.3 x 10 <sup>5</sup>
B2_16	4.0 x10 <sup>-3</sup>	6.7 x 10 <sup>5</sup>
B3_16	8.0 x10 <sup>-3</sup>	1.3 x 10 <sup>6</sup>
B4_16	5.0 x10 <sup>-3</sup>	8.3 x 10 <sup>5</sup>
B5_16	5.0 x10 <sup>-3</sup>	8.3 x 10 <sup>5</sup>
B6_16	7.0 x10 <sup>-3</sup>	1.2 x 10 <sup>6</sup>
D1_16	6.0 x10 <sup>-3</sup>	1.0 x 10 <sup>6</sup>
D2_16	3.0 x10 <sup>-3</sup>	5.0 x 10 <sup>5</sup>
D3_16	6.0 x10 <sup>-3</sup>	1.0 x 10 <sup>4</sup>
D4_16	2.0 x10 <sup>-3</sup>	3.3 x 10 <sup>5</sup>
D5_16	4.0 x10 <sup>-3</sup>	6.7 x 10 <sup>5</sup>
D6_16	BLD*	NA**
D7_16	8.0 x10 <sup>-3</sup>	1.3 x 10 <sup>6</sup>
D8_16	6.0 x10 <sup>-3</sup>	1.0 x 10 <sup>6</sup>
S1_16	BLD*	BLD
S2_16	4.3 x10 <sup>-2</sup>	7.2 x 10 <sup>6</sup>
S3_16	3.0 x10 <sup>-2</sup>	5.0 x 10 <sup>6</sup>
S4_16	2.8 x10 <sup>-2</sup>	4.7 x 10 <sup>6</sup>

\*BLD – Below limit of detection

\*\*NA – Not applicable. Lack of reading from the fluorimetry assay made the calculation of number of cells impossible.

#### 4.3.2 Bacterial and archaeal (16S) phylum level community composition

Bacterial 16S derived phyla distribution in the target basal ice types sampled from Svínafellsjökull and two other local glaciers (Skaftafellsjökull and Kvíarjökull) for comparison are presented in Figure 4.1. On average, the most abundant phyla were *Proteobacteria* ( $54.4 \pm 15.9$  %), *Acidobacteria* ( $8.9 \pm 5.6$  %), *Chloroflexi* ( $6.7 \pm 3.9$  %), *Actinobacteria* ( $6.1 \pm 4.8$  %), and *Nitrospirae* ( $6.5 \pm 10.3$  %). *Proteobacteria* was the dominant phylum among all basal ice samples, except for samples B1-15 where *Nitrospirae* (52.0 %) dominated. The second most abundant phylum was *Proteobacteria* (24.8 %). The phyla that appeared consistently among all samples were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Chloroflexi*, and *Cyanobacteria*.

Kruskal-Wallis analysis showed significant differences in some phyla between ice types: *Acidobacteria* (p-value = 0.003), *Cyanobacteria* (p-value = 0.03). *Acidobacteria* values were higher in debris band and stratified ice samples, but very low in dispersed facies. *Cyanobacteria*, on the other hand, were abundant in debris band and dispersed facies, but values were very low in stratified facies.

#### 4.3.3 Fungi, putative algae and plant (ITS) phylum level community composition

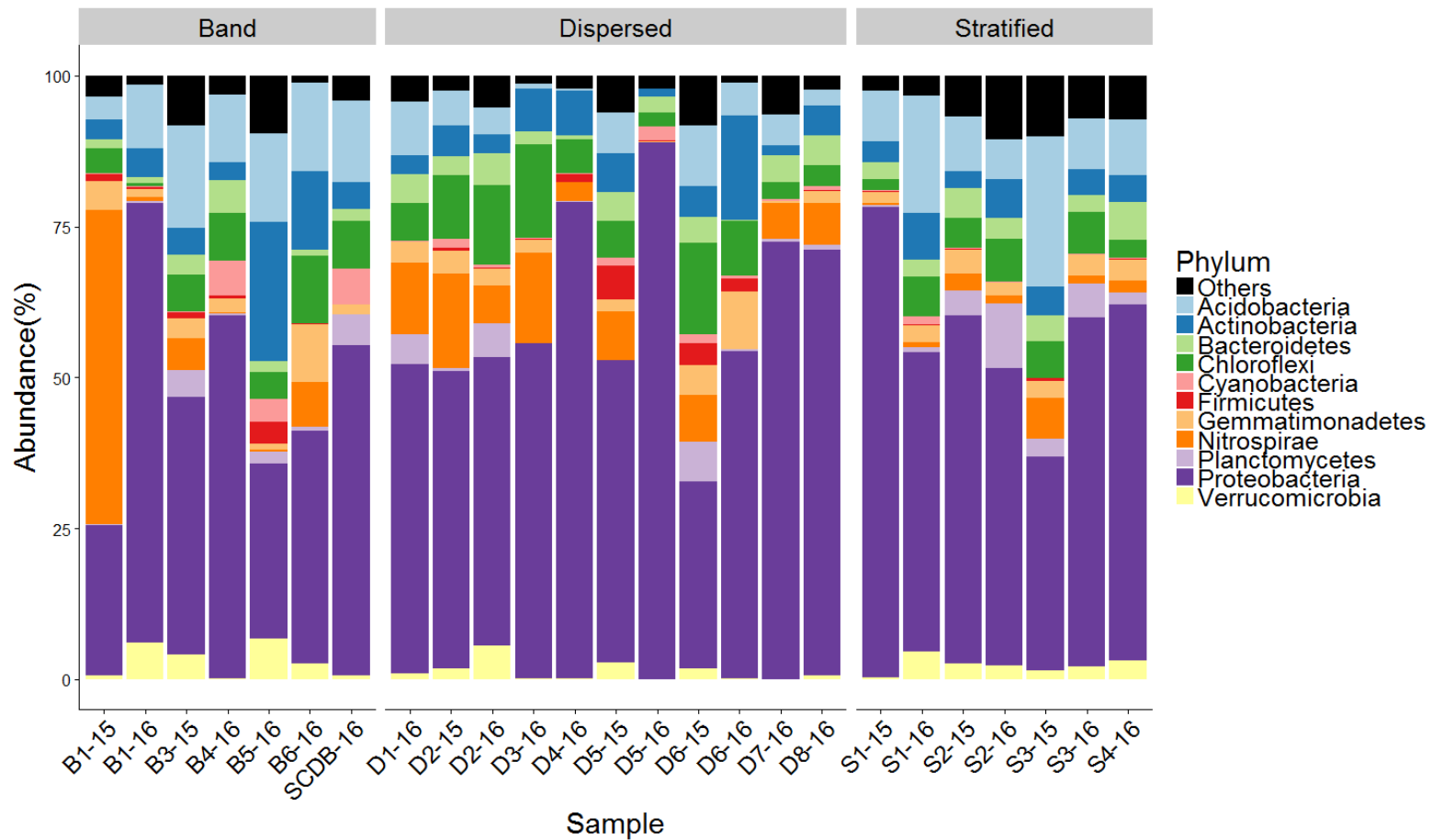
Figure 4.2 shows the analysis of the ITS sequences recovered from basal ice. Results showed that the most abundant fungal phylum was *Ascomycota* (Debris bands:  $42.5 \pm 38.3$  %, Dispersed:  $53.2 \pm 37.1$  %, Stratified:  $39.9 \pm 32.6$  %), followed by *Basidiomycota* (Debris band:  $18.1 \pm 31.6$  %, dispersed:  $18.0 \pm 31.7$  %, stratified:  $4.9 \pm 5.9$  %). *Chytridiomycota* was absent in debris bands and appeared in low abundance in stratified facies ( $0.9 \pm 2.4$  %) and dispersed facies ( $3.3 \pm 8.4$  %). *Glomeromycota* was absent in all samples. A significant number of plant-related sequences were found amongst the samples: *Chlorophyta* (debris band:  $0.6 \pm 1.6$  %, dispersed:  $3.8 \pm 11.8$  %, stratified:  $5.2 \pm 6.9$  %) and *Streptophyta* (debris bands:  $26.7 \pm 37.4$  %, dispersed:  $12.9 \pm 26.7$  %, stratified:  $17.8 \pm 21.2$  %). There were no statistically significant differences (p-value > 0.05) between basal ice types in terms of phyla distribution.

#### 4.3.4 Genera distribution among basal ice types

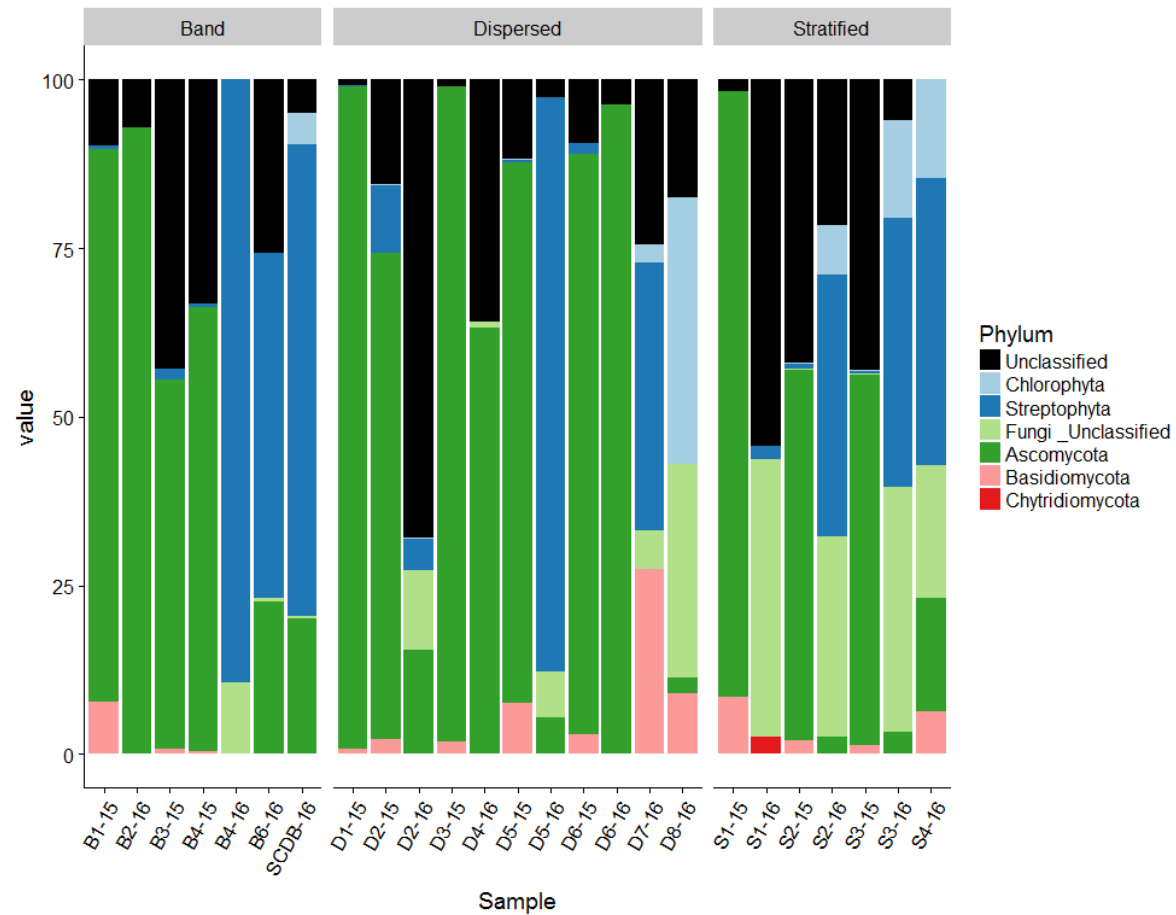
##### **Bacterial genera**

Bacterial genera-level distribution in each basal ice sample above the 2.5% abundance threshold, together with aggregated genera below the threshold, designated 'other genera', are presented in Figure 4.3. The unclassified category in Figure 4.3 refers to 16S sequences that did not yield any positive hits in the SILVA database (v. 123), built in Parallel-Meta 3.4.1. *Lysobacter* dominated in most of the samples, being more than 30% in D6\_15 and S1\_15, and GOUTA19 was well-represented in B1\_15 (28.5%). *Polaromonas* ranked as third in the most abundant genus, being particularly abundant in some dispersed facies samples (D7-16 – 20.1%, D8-16 – 14.6%, D4-16 – 13.5%). *Thiobacillus* appeared in most samples, except two dispersed facies samples (D5-16, and D6-16). Two groups that could not be resolved to genus level appeared to be abundant in some samples, such as FW\_4\_29 (16.2% in B1-15, 11.3% in D1-16) and GOUTA19 (28.5% in B1-15).

Significant (p-value <0.05) basal ice type-specific differences at the genus level are presented in Figure 4.4. Three genera showed statistically relevant differences. The first one is *Methylothera*, which in some dispersed facies samples was very abundant, but almost absent in debris bands and stratified facies samples. *Thiobacillus* showed high abundance in stratified ice, but was very low in debris bands, and intermediate values in dispersed. Similarly, to *Methylothera*, *Polaromonas* exhibited high variability in its abundance, but was very abundant in some dispersed facies samples, being almost negligible in stratified ice and debris bands (Figure 4.5)

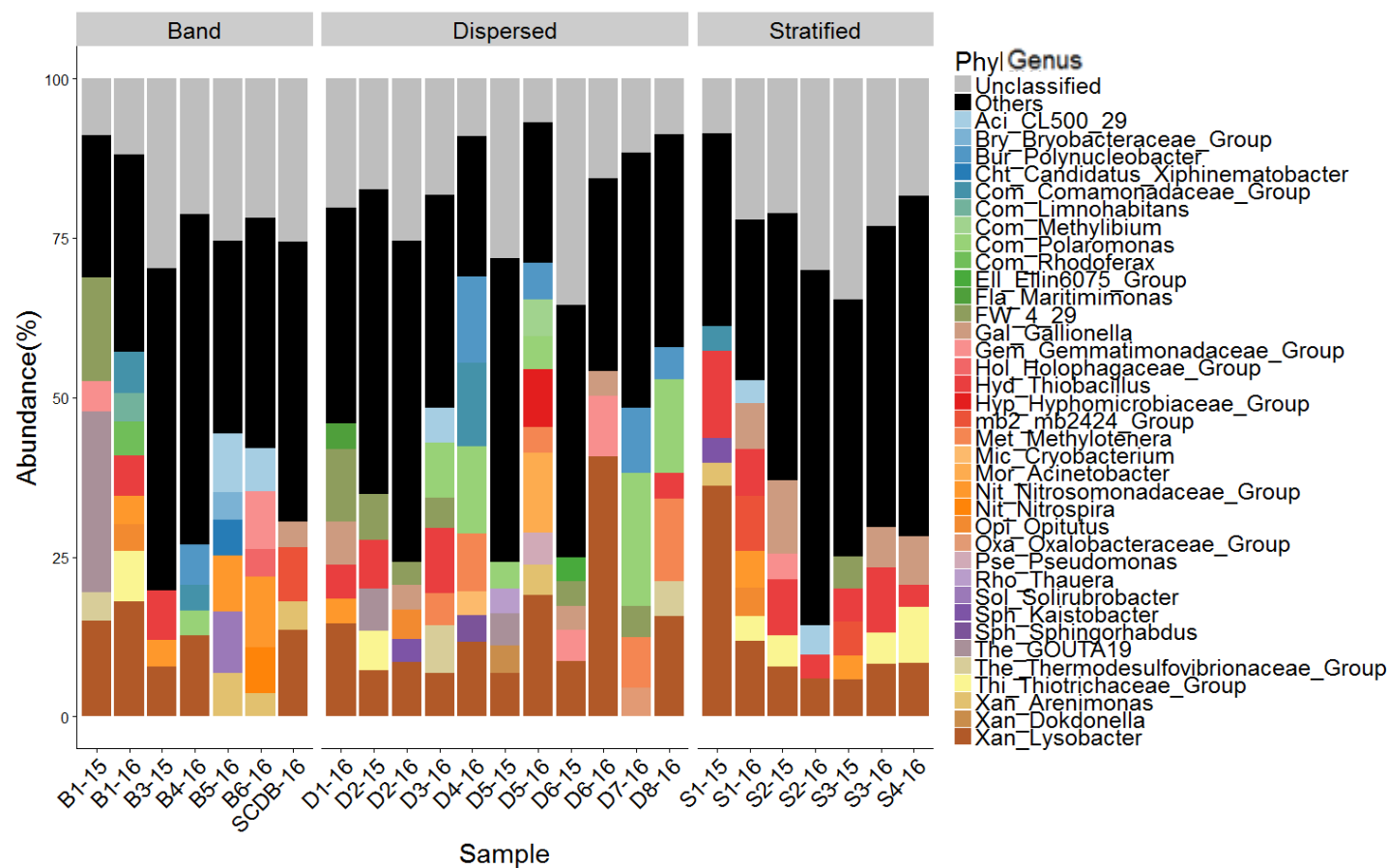


**Figure 4.1. Relative abundance of 16S rDNA sequences classified to phylum level in individual samples representing three basal ice types over the 2015 and 2016 sampling campaigns at Svínafellsjökull, Kvíarjökull and Skaftafellsjökull.** Based on 16S rRNA gene sequence derived OTU table analysis in Parallel Meta 3.4.1 of 16S rRNA. Samples labelled as B for debris bands, D for dispersed, and S for stratified. Samples from the 2015 sampling campaign are labelled as ‘\_15’ and from the 2016 campaign as ‘\_16’. All basal ice samples are from Svínafellsjökull, except B5\_16 and B6\_16 from Kvíarjökull, and D7\_16, 18\_16, and S4\_16, from Skaftafellsjökull glacier.

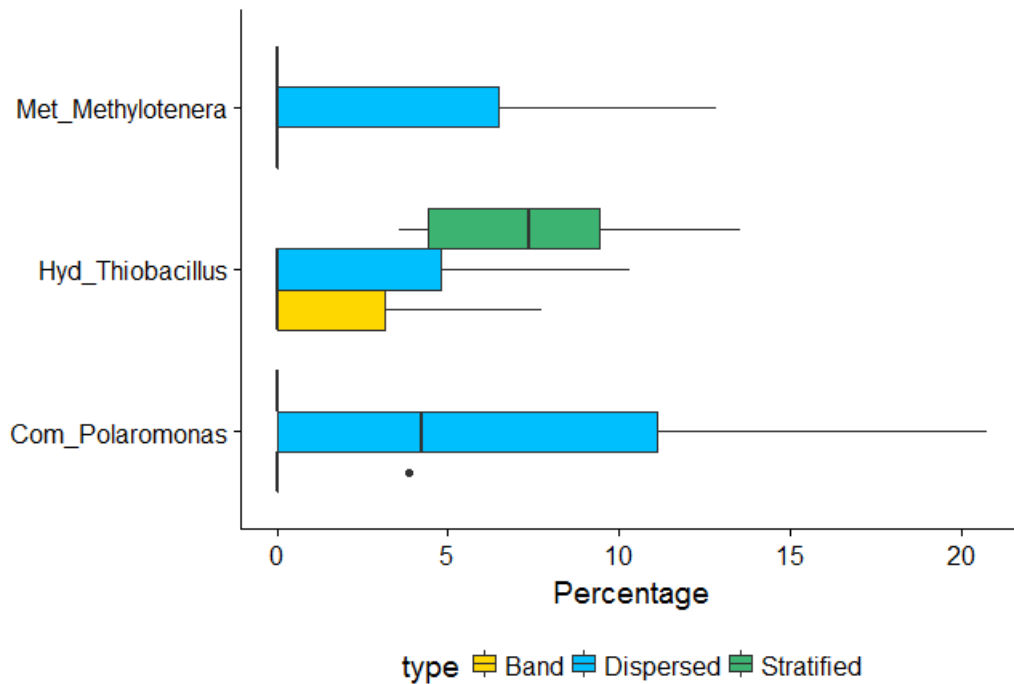


**Figure 4.2. Relative abundance of ITS (Internal Transcribed Spacer) sequences classified to phyla level in individual samples representing three basal ice types over the 2015 and 2016 sampling campaigns at Svínafellsjökull, Kviarjökull and Skaftafellsjökull.** Samples labelled as B correspond to debris bands, D dispersed, and S stratified. Samples from the 2015 sampling campaign are labelled as '\_15' and from the 2016 campaign as '\_16'. All samples are from Svínafellsjökull, except B5\_16 and B6\_16, which are from Kviarjökull, and D7\_16, 18\_16, and S4\_16, which are from Skaftafellsjökull.





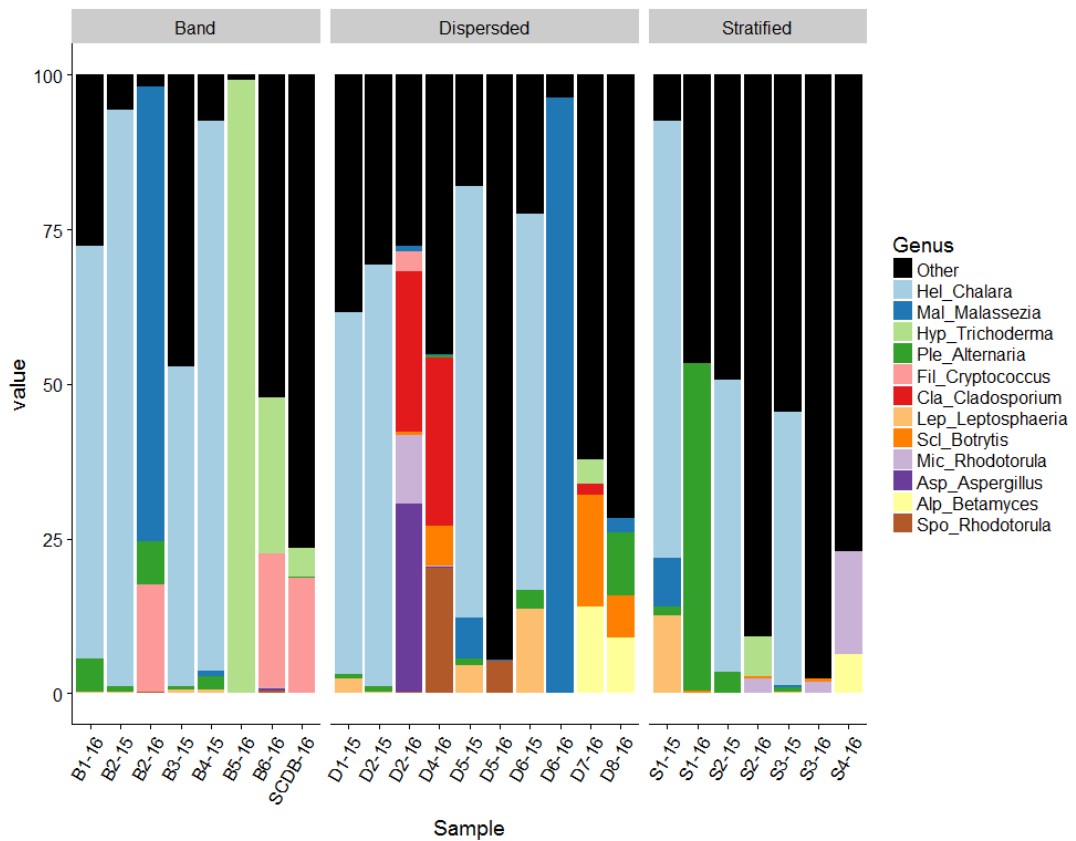
**Figure 4.3. Relative abundance of 16S sequences classified to genus level in individual samples representing three basal ice types over the 2015 and 2016 sampling campaigns at Svínafellsjökull, Kvíarjökull and Skaftafellsjökull.** Only groups that were more abundant than 2.5% were represented. Groups that were identified but below that threshold were grouped as “Others”. Sequences that did not return any hits were grouped as ‘unclassified’. Samples labelled as B correspond to debris bands, D dispersed, and S stratified. Samples from the 2015 sampling campaign are labelled as ‘\_15’ and from the 2016 campaign as ‘\_16’. All samples are from Svínafellsjökull, except B5\_16 and B6\_16, which are from Kvíarjökull, and D7\_16, D8\_16, and S4\_16, which are from Skaftafellsjökull.



**Figure 4.4. Differential bacterial genera by ice type.** Bacterial genera that showed statistically significant differences (Kruskal-Wallis p-value < 0.05) between ice facies were represented in box plots. Box limits show 25<sup>th</sup> and 75<sup>th</sup> percentiles of the data, whiskers extend to 5<sup>th</sup> and 95<sup>th</sup> percentiles and dots represent the outliers.

### Fungal genera distribution

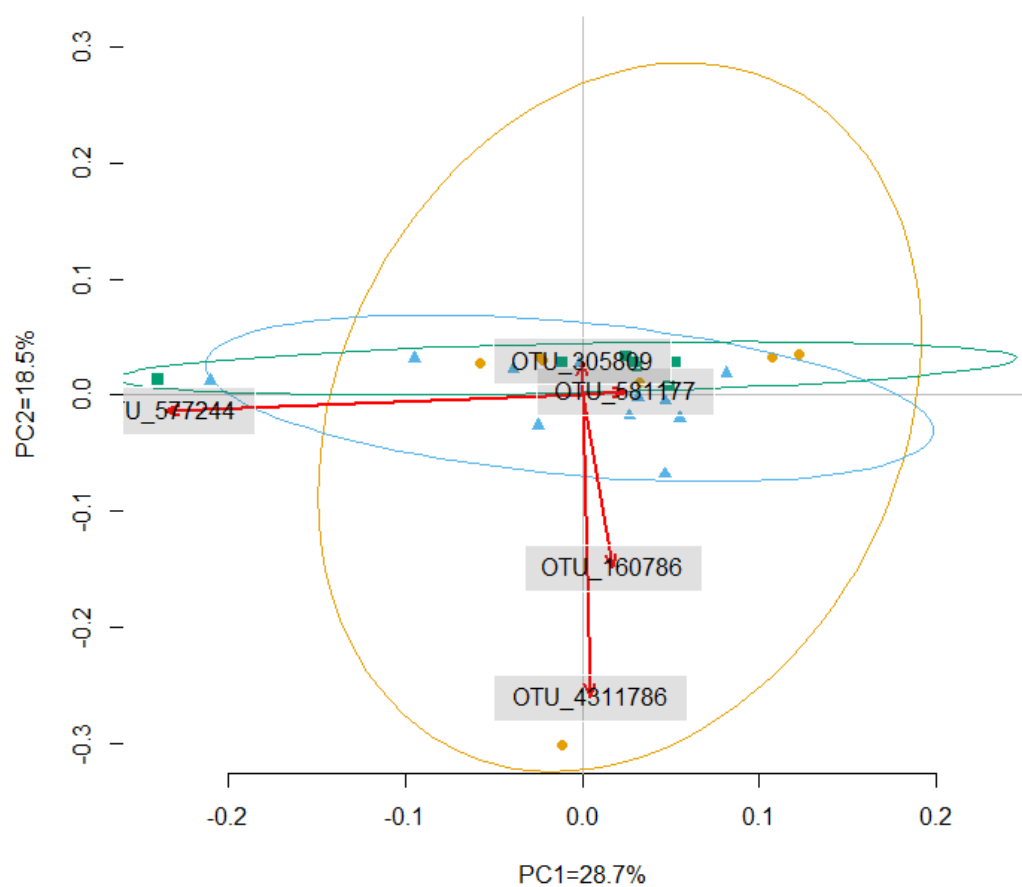
The dominant fungal genera are represented in Figure 4.5. The most abundant genus is *Chalara* (debris band: 22.3±31.0%, dispersed: 35.5±41.6%, stratified: 23.2±30.1%) *Trichoderma* (debris band: 21.2±36.4%, dispersed: 0.6±1.9% , stratified: 1.7±4.6%), *Cryptococcus* (debris band: 17.0±27.9%, dispersed: 0.3±1.1% , stratified: 0.0±0.0%), *Alternaria* (debris band: 1.6±2.7%, dispersed: 2.0±4.9 , stratified: 8.1±20.3% ), *Cladosporium* (debris band: 0.0±0.0%, dispersed: 5.4±11.1% , stratified: 0.0±0.0%). However, unlike the bacterial genera, there were no statistically significant differences between basal ice type samples (Kruskal-Wallis p-value > 0.05).



**Figure 4.5. Relative abundance of ITS sequences classified to genus level in individual samples representing three basal ice types over the 2015 and 2016 sampling campaigns at Svínafellsjökull, Kvíarjökull and Skaftafellsjökull.** Only groups that were more abundant than 1% were represented. Groups that were identified but below that threshold were groups as “Other”. Sequences that did not return any hit were groups as unclassified. Samples labelled as B correspond to debris bands, D dispersed, and S, stratified. Samples from the 2015 sampling campaign are labelled as ‘\_15’ and from the 2016 campaign as ‘\_16’. All samples are from Svínafellsjökull, except B5\_16 and B6\_16, which proceed from Kvíarjökull, and D7\_16, 18\_16, and S4\_16, which proceed from Skaftafellsjökull

#### 4.3.5 Bacterial OTU distribution by ice type

Overall, 10162 different OTUs were delineated applying a 97% similarity cut-off. Bacterial community (OTU) distribution in relation to ice types following Principal Component Analysis is presented in Figure 4.6. Based on 95% confidence ellipses, no statistical differences in bacterial communities in relation to ice types were detected. Stratified basal ice communities showed the tightest clustering compared to communities originating from dispersed facies and debris band. The most relevant OTUs were plotted in a vector analysis and identified in the biplot (Fig. 4.6). Identities (BlastN) of significant vectored OTUs are presented in Table 4.



**Figure 4.6. Principal Component analysis (PCA) of bacterial communities constrained basal ice types (Dispersed, Stratified, Debris band) at Svínafellsjökull.** PCA based on relative abundance of 10162 Operational Taxonomic Units (OTUs) across the three basal ice types. Markers indicate basal ice type: orange – debris bands, blue – dispersed facies, and green – stratified. Dispersion ellipses show 95% confidence interval for each basal ice type. Significant ( $p < 0.05$ ) OTUs within bacterial communities are bi-plotted as vectors.

**Table 4.3. Bacterial identities of significant vectored bi-plotted bacterial (p <0.05) Operational Taxonomic Units from basal ice-specific bacterial communities following multivariate ordination (Principal component analysis).**

OTU ID	Query homology	Parallel-Meta result	Source of sample	BlastN result <sup>#</sup>	GenBank accession number	Most similar cultured species <sup>~</sup>	Source of isolate	S_ab score	RDP accession number
577244	98%	<i>Lysobacter</i>	Glacier front	Uncultured bacterium	JF715971.1	<i>Lysobacter ginsengisoli</i>	Soil	0.900	AB245363
160786	97%	FW; 4_29	Permafrost	Uncultured bacterium	JX981781.1	<i>Nitrospira marina</i>	Corroded iron pipe	0.611	X82559
4311786	89%	GOUTA19	Sediment aquifer	Uncultured bacterium	JX120395.1	<i>Nitrospira marina</i>	Marine sediment	0.548	JQ073799
581177	99%	CL500_29	Basalt aquifer	Uncultured bacterium	KX163790.1	<i>Ilumatobacter fluminis</i>	Estuary sediment	0.639	AB360343
305809	99%	<i>Polynucleobacter</i>	Arctic stream	Uncultured bacterium	FJ849309.1	<i>Polaromonas rhizosphaerae</i>	Soil	0.966	EF127651

\* Significant biplotted vectored OTUs (Figure 4.6).

<sup>#</sup>BlastN/GenBank search

<sup>~</sup>RDP search

## 4.4 Discussion

### 4.4.1 Microbial community diversity of basal ice

#### *Bacterial and archaeal (16S) diversity*

Major phyla identified in basal ice types investigated were *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Actinobacteria* and *Nitrospirae*, but only *Proteobacteria* and *Acidobacteria* appeared consistently in all samples (Fig 4.1).

*Proteobacteria* represents one of the largest phyla in the domain Bacteria and encompasses a very complex assemblage of physiologies, including phototrophy, heterotrophy, and chemolithotrophy (Gupta, 2000). *Acidobacteria* are a highly diverse and abundant Phylum resident in a wide range of habitats (Kielak et al., 2016). Previous research showed, as a general rule, that the abundance of *Acidobacteria* increases in soils as pH falls (Sait et al., 2006), and in cold environments in particular, such as cold soils or glaciers (Lipson and Schmidt, 2004; Männistö et al., 2012). Members of this group have been suggested to be oligotrophic, not only due to the fact that they thrive in low C environments, but also because members of the phylum *Acidobacteria* only present 2 copies of the 16S rRNA gene, suggesting low growth rates and affiliation to K-strategists, microorganisms adapted to low nutrient concentrations, showing high substrate affinity (Fierer et al., 2007). These data clearly support the general view that basal ice represents an oligotrophic and acidic environment as has also been identified in the geochemistry data of this study (Chapter 3) and in data from other glaciers (Skidmore et al., 2005; Mader et al., 2006). Previous studies have suggested that *Acidobacteria* and *Proteobacteria* have ecological inter-relationships since they are often found to dominate diverse environmental microbiomes (Kielak et al., 2016). Several members of the *Proteobacteria* group are chemolithotrophs, and *Acidobacteria* are chemo-organotrophs that can survive on autotrophically fixed C in poorly oxygenated environments. Subglacial environments have been shown to support spatially heterogeneous anaerobic pockets (Wadham et al., 2007), which would explain the co-occurrence of these two phyla (Kielak et al., 2016).

*Chloroflexi* is a ubiquitous bacterial Phylum, which have been found in a variety of environments, such as activated sludge, thermal springs, hypersaline mats, and

water-saturated cold soils (Björnsson et al., 2002; Costello and Schmidt, 2006). *Chloroflexi* encompass different bacterial metabolisms: anoxygenic photoautotrophs, anaerobic dehalogenic reducers, aerobic, microaerobic, and anaerobic organotrophs, ranging from mesophiles to thermophiles (Campbell et al., 2014), but they have also been found in cold environments (Costello and Schmidt, 2006).

*Actinobacteria* constitutes another major bacterial phylum that inhabits a wide range of terrestrial and marine environments. Members of this phylum are known to produce a wide variety of extracellular enzymes and secondary metabolites (Ventura et al., 2007). Most members of this phylum are heterotrophs, with some exceptions (Barka et al., 2016), such as the chemoautotroph *Acidimicrobium ferrooxidans* (Norris et al., 2011). *Nitrospirae* is a phylum that contains chemolithotrophic nitrite-oxidising bacteria, dissimilatory sulphate reducers, and magnetotactic bacteria (Iguchi et al., 2009).

Among the samples, sequences affiliated with *Euryarchaeota* were identified in 16 out of 24 samples, being of significant abundance in D5-15 (4.7%). The presence of microorganisms affiliated with the taxon is highly relevant, since this phyla harbours methanogenic archaea that, in anaerobic conditions, can generate methane (Zhang et al., 2008; Boyd et al., 2010; Wadham et al., 2012; Dieser et al., 2014).

A more in-depth study, resolving down to genera level (Fig 4.3), showed that the most abundant genera were *Lysobacter*, *Thiobacillus*, *Gallionella*, *Polaromonas*, *Methylothermus*, *Rhodoferrum*, *Acidovorax*, *Methylobium*, *Kaistobacter*, and *Maritimonas*. Other major families that could not be resolved to genus level were *Nitrosomonadaceae*, *Gemmatimonadaceae*, *Comamonadaceae*, FW\_4\_29, *Thiotrichaceae*, CL500\_29, mb2424, and GOUTA19.

Some of the dominant taxa contain well-known chemolithoautotrophic genera: *Thiobacillus* and *Gallionella*, contains some Fe-oxidising species (Trudinger and Swaine, 1979), and the families *Comamonadaceae* (such as *Hydrogenofaga*, *Rhodoferrum*, hydrogen-oxidisers) and *Thiotrichaceae* (*Thiotrix* or *Beggiatoa*, sulphur-oxidisers) (Bertrand et al., 2015). Interestingly, *Thiobacillus* was one genera that showed statistical differences between ice facies, being particularly abundant in

stratified facies. The presence of individuals belonging to these taxa is highly relevant in relation to the physico-chemical analyses of the basal ice at Svínafellsjökull. The low carbon content, and a lack of light, would strongly select for chemolithotrophy-based primary production typical of related environments (Parnell et al., 2002; Tyler et al., 2002; Domack et al., 2005). Interestingly, *Thiotricaceae*, which contains some of the best characterised S-oxidisers (Bertrand et al., 2015), were abundant in sediment-rich basal ice (debris bands and stratified ice) (Figure 6), which suggests that sulphur cycling would play a major role in the primary production in these types of ice (Stibal, Tranter, Benning, et al., 2008; Boyd et al., 2014). The presence of active chemolithotrophs would mean an input of C that could be used by associated heterotrophic bacteria. Some of the heterotrophic genera found were *Lysobacter*, *Polaromonas*, *Methylothera*, *Rhodoferrax*, *Acidovorax*, *Methylibium* and *Kaistobacter*. *Lysobacter* is a heterotroph known for its ability to produce a battery of enzymes that makes it able to attack bacterial competitors and cause lysis (Christensen and Cook, 1978; de Bruijn et al., 2015). OTUs affiliated with *Lysobacter* are one of the biplot vectors in Fig 4.6, being almost parallel to the horizontal axis, which would indicate that the presence of this genus is highly important. Cell lysis releases nutrients to the environment supporting growth of other microorganisms, and it is well known that is a driving factor in nutrient cycling in cold environments (Edwards et al., 2006; Bellas et al., 2013). *Polaromonas*, as the name implies, is a widely distributed genus in cold environments with a very versatile metabolism (Darcy et al., 2011), and it was one of the group that showed significant differences between ice types, showing high abundance in dispersed facies in particular. Sequences associated with the genus *Kaistobacter* have been identified, and sequences affiliated with this genus have also been found in subglacial environments, specifically in subglacial sediment (Stibal et al., 2012). Methylotheric genera have also been found in the investigated basal ice. These bacteria are able to consume methane and other C1 compounds, and are considered chemolithotrophs as a consequence. They can be either strict methylotherics, such as *Methylothera*, or facultative, such as *Methylibium*. *Methylothera* is able to consume methylamine as C and N source, and if only methanol is provided, it reduces nitrate (Mustakhimov et al., 2013). It was one of the groups that showed statistically significant differences in



their abundance, being abundant in dispersed ice. *Methylibium*, on the other hand, can use both C1 and multi-carbon compounds, and under anaerobic condition is able to reduce nitrate to nitrite (Stackebrandt et al., 2009). Methanotroph *Methylibium*-affiliated sequences have been found in subglacial sediments (Boyd et al., 2014). *Maritimimonas*, on the other hand, is a genus that comprises aerobic organisms isolated from marine environments (Park et al., 2009).

Although resolved to family but not genus levels, several sequences affiliated with the family *Nitrosomonadaceae* were identified. This family contains lithoautotrophic members, able to oxidise ammonia, generating  $\text{NO}_2^-$  that can be used subsequently to generate  $\text{NO}_3^-$  (Prosser et al., 2014). Stratified ice contained a high abundance of *Nitrosomonadaceae* members (Figure 4.3), which suggests that, apart from sulphur cycling, ammonia oxidation plays a very important role in the primary production in this type of ice. The families *Gemmatimonadaceae* and *Comamonadaceae* respectively include heterotrophic aerobic species (Zhang et al., 2003) and a very varied membership that includes denitrifying bacteria (Khan et al., 2002), and in this study, were significantly more abundant in dispersed ice and debris bands. The latter family also includes other of the genera also identified in this work, namely *Polaromonas*, *Acidovorax* or *Rhodoferrax*. *Acidovorax* is a genus that comprises several plant-pathogenic species. However, some of its members are able to reduce nitrite and oxidise Fe(II) (Klueglein et al., 2014). *Rhodoferrax* genus contains members that are facultative anaerobes with the capacity of reduce Fe(III) in their respiration (Finneran et al., 2003). Some other *Rhodoferrax* are fermenters.

GOUTA19 belongs to the *Thermodesulfobibrionaceae* family, which contains sulphate-reducing species, and sulphate values were significant in the ice matrix, and sulphate containing minerals were found in the SPA. This group was highlighted by one of the vectors in the biplot (Fig 4.6), which pointed towards where the majority of the samples with high sulphate levels, thereby highlighting the importance of sulphate in the basal ice environment. Although the most sequences found in the BlastN search showed only 89% similarity, it is interesting that they were found elsewhere in the sediment of an aquifer (Accession number: JX120395.1), which is an oligotrophic sediment-rich environment, with obvious similarities to basal ice. Little

information is available on other identified taxa, such as FW\_4\_29, CL500\_29, mb2424, and EM3.

#### 4.4.2 Co-occurrence

Fig 4.7 shows the co-occurrence and co-exclusion of genera based on the 16S rDNA analysis. In general, network analysis showed that there is a correlation of heterotrophs and autotrophs, which supports the hypothesis that as basal ice represents an oligotrophic environment, C-input via chemoautotrophic carbon fixation is essential in order to maintain microbial productivity under dark anaerobic conditions (Chapelle et al., 2002; Parnell et al., 2002; Tyler et al., 2002; Momper et al., 2017).

*Lysobacter* co-occurs with *Kaistobacter*, *Sporichthyaceae*, and *Staphylococcus*. *Kaistobacter* is a genus that has been found in temperate (Lopes et al., 2014; Wu et al., 2017) and cold (Yang et al., 2016) soils. *Sporichthyaceae* is family of heterotrophs (Tamura et al., 1999) that belongs to the Actinobacteria phylum, and members of this genus have been identified in cold soils in Antarctica (Babalola et al., 2009). Little information is available for ecological interpretation about this group regarding how fastidious its culturing is (Normand, 2006). Members of the genus *Staphylococcus* from icy environments have been reported consistently in the literature (Zhang et al., 2001, 2002; Miteva et al., 2004). This cluster seems to be formed mainly by heterotrophs, which would mean that they could survive in either C that had been entrained by the glacier in its advance (Ives, 2007) or once chemolithotrophs have fixed C and the minerals are completely oxidised, therefore no longer suitable for the presence of chemolithotrophs.

*Thiobacillus* which comprises chemolithotrophic sulphur-oxidising bacteria (Hutt et al., 2017) co-occurs with *Leeia* (aerobic heterotrophs with the ability to reduce nitrate (Lim et al., 2007)) and with RB40 groups, a bacterial group that has been found in soils (Sessitsch et al., 2001).

One of the clusters is formed by two potential chemolithotrophs: *Polaromonas*, which can facultatively oxidise H<sub>2</sub> and Fe(II) (Jeon et al., 2004; Yagi et al., 2009; Darcy et al., 2011); and *Methylothermus*, able to oxidise C<sub>1</sub> compounds (Mustakhimov et al.,

2013); and a heterotroph, *Polynucleobacter*, a cosmopolitan widely distributed genus. The three of them co-occur with *Sphingorhabdus*, aerobic heterotrophs that have been isolated from sea waters and sandy sediments (Jogler et al., 2013; Romanenko et al., 2015); and *Acidovorax*, another autotrophic genus able to oxidise  $H_2$  (Lee et al., 2015) or Fe(II) (Pantke et al., 2012) in microaerophilic environments, while respiring nitrate. Both *Polaromonas* and *Methylothera* co-occurs with other heterotrophs: the strictly aerobic *Ferruginibacter* and the facultatively anaerobic *Simplicispira* able to reduce nitrate to nitrite or  $N_2$  and some of them can grow at low temperatures (Grabovich et al., 2006; Lu et al., 2007; Hyun et al., 2015). *Polaromonas* and *Methylothera* co-occur with *Oxolobacteraceae*, a metabolically diverse family, which includes aerobes, anaerobes, and nitrogen fixing organisms, (Baldani et al., 2014), roots colonisers (Ofek et al., 2012), and some members have been isolated from cold environments, such as glacier forelands (Bajerski, Ganzert, Mangelsdorf, Lipski, et al., 2013) or cryoconite (D.-C. Zhang et al., 2011). *Polaromonas* also co-occurs with *Desulfuromonas*, strict anaerobes able to use Fe(III), S, Mn (III) as TEA and oxidising organic compounds, such as acetate (Pfennig and Biebl, 1976; Gebhardt et al., 1985). Whilst *Desulfuromonas* reduces Fe(III) in anaerobic conditions as TEA, *Polaromonas* can use the reduced Fe(II) to generate organic matter, that can be used by the former genus. *Polynucleobacter* co-occurs with *Cryobacterium* (aerobic heterotrophs with psychrophilic members isolated from glaciers, glacial and Arctic soils (Suzuki et al., 1997; Reddy et al., 2010; Liu et al., 2012)).

*Gallionella* (strict aerobes that are S- and Fe-oxidisers (Kucera and Wolfe, 1957; Jin et al., 2017) (Hallberg et al., 2011) and *Thiotrichaceae* (autotrophic S-oxidisers (Bertrand et al., 2015)) are part of the same cluster, both of them co-occurring with *Parafilimonas* (aerobic heterotrophs (Kim et al., 2014), *Nitrosospora* (another chemolithotrophic genera, able to oxidise  $NH_4^+$  to  $NO_2^-$ ) (Klotz et al., 2006), and *Sideroxydans* aerobic (Fe(II) oxidising chemolithotrophs. *Gallionella* also appears linked to *Maritimonas* (aerobic heterotrophs (Park et al., 2009)). *Thiotrichaceae*, on the other hand, appears linked to *Opitutus* (strict aerobic fermenters, generating propionate and acetate as a result (Chin et al., 2001)), *Haliangium* (aerobic heterotrophs (Fudou et al., 2002)), *Rhodoferax* (members of this group can grow

autotrophically using H<sub>2</sub> (Finneran et al., 2003)) , and *Cytophagaceae* (a very wide family of the heterotrophs (McBride et al., 2014) some of which can fix N (Xu et al., 2014), and also isolated from cold environments (Jiang et al., 2013).

*Nitrosomonadaceae* is family which includes ammonia oxidisers (Prosser et al., 2014) appears with *Nitrospira* and *Candidatus Nitrotoga* (which is able to oxidise the NO<sub>2</sub><sup>-</sup> produced by member of *Nitrosomonadaceae*). *Nitrosomonadaceae* also co-occurs with *Holophagaceae* (family of heterotrophs, some of which are fermenters (Fukunaga and Ichikawa, 2014)), and CL500\_29, and *Bryobacteraceae* (family which comprises aerobic and facultative heterotrophs (Dedysh et al., 2017)).

FW-4-29 (belongs to *Nitrospirales* order) and GOUTA19 (belongs to the *Thermodesulfovibrionaceae*) appear together, in association with *Smithella* (strict anaerobe able to oxidise propionate and formate, in the presence of a methanogenic partner), and BD2\_6. This last group is affiliated with the *Thermodesulfovibriales* order.

Interestingly, most of the chemolithotrophic groups occupied the central part of the network analysis and appeared connected (e.g. *Candidatus Nitrotoga-Nitrosomandaceae-Nitrospira*, *Gallionella-Sideroxydans-Acidiferrobacter*, *Gallionella-Thiotrichaceae-Nitrosospira*). This could suggest that chemolithotrophic species would colonise C-depleted environments and, due to their actions, enrich it in C, allowing colonisation by heterotrophs. Heterotrophic groups would thrive once the C levels increase allowing heterotrophic metabolism.

Some aerobic groups co-occur with anaerobes, such as *Desulforomonas*. It co-occurs with *Ferruginibacter*, which is a strict aerobe, and *Simplicispira* (facultative anaerobe), and with *Polaromonas*, that have very versatile metabolisms. This kind of relationship would suggest that within the basal ice, the microbial communities are organised as biofilms. Whilst autotrophic individuals, such as *Polaromonas*, fix C into organic matter for the biofilm, aerobes, such as *Ferruginibacter*, would appear in the surface of the biofilm where oxygen is present. Intermediate positions could be occupied by *Simplicispira*, which can tolerate oxygen, but also be active in its absence

reducing nitrate. The innermost part of the biofilm would be occupied by the anaerobic individual - in this case, *Desulforomonas*.



#### 4.4.3 ITS diversity

ITS sequence data showed a high abundance of plant-related sequences, belonging to *Streptophyta* and *Chlorophyta* phyla (Fig 4.2). The phylum *Chlorophyta* is a taxa of algae that have been found in supraglacial ecosystems (Remias et al., 2009; Stibal, Šabacká, et al., 2012) and members of this phyla have been isolated from subglacial ecosystems, where they were suggested to be latent, and might be playing very important roles in soil formation and primary colonisation once released from the glacier and exposed to light (Kaštovská et al., 2005, 2007). *Streptophyta* (Remias et al., 2012) comprises embryophytes and vascular plants, as well as a group of green algae. No plant-derived matter was visually identified during the sampling, but presumably the presence of plant-derived DNA proceeds from entrainment as the climate in Iceland became cooler, between the the 14<sup>th</sup>-19<sup>th</sup> centuries (Ives, 2007). Svínafellsjökull's foreland was used for agricultural purposes, so it is likely that during the glacier advance, the plant material was entrained into basal ice at the same time as sediment (Ives, 2007). In addition, the frozen conditions of the subglacial environment would preserve the DNA as relic DNA. The majority of non-fungal ITS sequences belonged to the family *Chlamydomonadaceae* and the genus *Raphidonema*, *Sonchus* and *Rhododendrum*. Other plant-related sequences were found in the ITS analysis, such as *Bryum*, which is a genus of mosses that have been found in Iceland and that are considered to be colonising species (Ingimundardóttir et al., 2014), and *Trifolium*, and individuals belonging to this genus have been found in Iceland. The family *Chlamydomonadaceae* and the genus *Raphidomona* comprises algae that have been found in cold environments (snow) (Hoham, 1973, 1980; Stibal and Elster, 2005). Members of both *Chlamydomonadaceae* and *Raphidonema* have been found in Icelandic glaciers (Lutz et al., 2015), and *Raphidonema* was particularly abundant in Vatnajökull snow.

Fungal sequences affiliated with the phyla *Ascomycota*, *Basidiomycota*, and *Chytridiomycota* were identified for the first time in glacier basal ice (Fig 4.2). *Ascomycota* has been shown to be the dominant phylum in recently deglaciated soils, whereas *Basidiomycota* is the most abundant phyla in old soils (Zumsteg et al., 2012; Brown and Jumpponen, 2014). *Ascomycota* comprises lichen-forming species, which

are highly important in primary colonisation in deglaciated sediment (Zumsteg et al., 2012). W. Zhang et al., (2011) also found that the dominant phyla in Chinese glaciers were *Ascomycota* and *Basidiomycota* and suggested that these two phyla are adapted to cold temperatures. In addition, they suggested that fungi in cold ecosystems could degrade organic matter, helping the recycling of nutrients (W. Zhang et al., 2011). This process would be of critical importance in basal ice, since the C content was found to be very low. Furthermore, as mentioned earlier, Svínafellsjökull overrode agricultural land (Ives, 2007), and if plant material was entrapped in the basal ice, as the presence of plant-related species indicates, the decomposition of such plant-derived organic matter would be highly important to release nutrients that could be subsequently used by the microbial community. Individuals closely affiliated with the sequences found in basal ice at Svínafellsjökull have been recovered from ice: *Cladosporium* and *Alternaria* can grow at low temperatures and under low oxygen tensions (Ma et al., 2000), which are conditions likely to occur in basal ice of Svínafellsjökull, and have been recorded in other glaciers (Bottrell and Tranter, 2002; Wadham et al., 2007; Stibal, Hasan, et al., 2012). Members from the genus *Rhodotula* have also been found in cryoconite holes in Svalbard, and in Austrian glaciers (Edwards, Douglas, et al., 2013). *Chytridiomycota* comprises aquatic fungi able to decompose plant matter but also able to parasitise algae. Interestingly, sequences affiliated with this genus were not found either in Svínafellsjökull or Kvíárjökull, but were present at Skaftafellsjökull (Figs 4.3 and 4.4). *Glomeromycota* is one the widest distributed fungal phyla, but sequences affiliated with this phylum were not identified, although the SSU (18S) is the marker of choice (Opik et al., 2011). *Glomeromycota* is known to establish symbiotic relationships with plants, generating mycorrhiza (Schüßler et al., 2001). The conditions in basal ice prevent the growth on any kind of plant, impeding the establishment of *Glomeromycota* individuals in the basal ice, which could explain the lack of sequencings of this phylum among the samples. Most fungal isolates from ice correspond to yeasts species, followed by yeast-like species (Cantrell et al., 2011). This could be explained by the nature of ice, in which space is very limited, and require that the microbial species surviving in these conditions are adapted to restricted liquid water spaces. At ~0°C, brine vein diameters are ~10 µm, which can



accommodate filamentous fungi and single celled yeasts, but this value varies with temperature (Mader et al., 2006).

The most abundant genus was *Chalara*, which includes saprotrophic litter decomposers but also the plant pathogenic species *Chalara fraxinea*, which is raising concerns in Europe as being the causal agent responsible for ash dieback (Gross et al., 2016). Previous research has found a high abundance of plant-pathogenic fungi entrapped in sediment within ice, possibly protected in plant tissues and particularly roots (Rime et al., 2016). The second most abundant genus was *Trichoderma*, which are known to be free-living fungi that can sometimes establish symbiotic relationships with plants. They have been isolated from cold soils in glacier forelands from the Himalayas (Ghildiyal and Pandey, 2008) and the Swiss Alps (Brunner et al., 2011), and some of them are able to grow at low temperatures. Interestingly, members of the genus are known to produce metabolites with the ability to inhibit the growth of other fungi. Some of them have also been found in this study, such as *Alternaria* and *Cladosporium*. When temperatures fall below 4°C, some member of the genus *Trichoderma* can sporulate, allowing it to survive in harsh conditions (Ghildiyal and Pandey, 2008). *Cryptococcus* is a genus that comprises psychrotolerant yeasts able to grow at temperatures as low as 4°C and have been found in cold ecosystems, such as in glaciers of the Pakistani Himalayas (Hassan et al., 2017), and snowpack and Antarctic soils (Dreesens et al., 2014). *Cryptococcus* have been suggested to play a very important role in the decomposition of algal organic matter, facilitating the recycling of nutrients into the ecosystem (Adams et al., 2006; Buzzini et al., 2012), and DNA associated with algal phyla have been found in this work. Members of the genus *Cryptococcus* have also being identified in Greenland (Ma et al., 1999) and Vostok ice cores (Abyzov, 1993). *Cryptococcus* was not the only genus of yeasts found in this work as members of the genus *Rhodotula* were also identified. *Alternaria* is a fungal genus with species isolated in cold environments such as Antarctica (Adams et al., 2006). Members of the genus *Cladosporium* have been found in cold environments, and they have been recovered from glacier ice cores (Ma et al., 2000); although they are not strictly yeasts, they are considered yeast-like fungi (Cantrell et al., 2011).

The high abundance of sequences affiliated with yeast and yeast-like genera could be explained by the size requirement in order to survive within basal ice. The ability to survive as small single cells would be crucial in order to thrive under these conditions, and some of them have sound adaptations to cold temperatures, such as increased membrane fluidity and the production of osmoprotectants (Buzzini et al., 2012).

#### 4.4.4 Chemolithotrophy and mineral weathering in basal ice

Subglacial environments in general, and basal ice in particular, are known to be oligotrophic (Skidmore et al., 2000; Foght et al., 2004). In these conditions, mineral weathering plays a fundamental role, since weathering mobilises nutrients and makes them bioavailable, which can be either beneficial or detrimental for the microorganisms inhabiting the environment (Uroz et al., 2009). Svínafellsjökull is situated over basaltic bedrock, and some studies have shown that microorganisms can weather basalts, obtaining energy from the oxidation of sulphates, methane, ferrous iron, manganese and hydrogen, as well as reducing ferric iron, sulphate, and CO<sub>2</sub> for respiration (Dong, 2010). Through production of organic acids, fungi are able to weather basalts and other igneous rocks (Hoffland et al., 2004).

In order to maintain an active ecosystem in the basal ice, there is a need for C input by C-fixation. In dark environments, such as basal ice, where photosynthesis is inhibited, the entry of C into the ecosystem is associated with chemolithotrophy and several chemolithotrophic taxa-related sequences were found in this study. In addition, living cells require electron donors and acceptors, macronutrients, such as N and P, and to a lesser extent Fe, as well as different micronutrients depending on the microorganism and the habitat (Rogers and Bennett, 2004). Apart from chemolithotrophy, microorganisms can interact with minerals in different ways, such as oxygen-independent respiration where other terminal electron acceptors can be used, which leads to the reduction of a range of molecules. These can be either soluble (i.e. nitrate, sulphate) or insoluble, as part of minerals, requiring direct contact between the microorganism and the mineral surface. Microorganisms including fungi produce acidifying substances, such as protons or organic molecules (Welch & Ullman 1993, Hoffland et al., 2004), which can dissolve certain minerals, such as carbonates. In addition, microbial respiration can generate CO<sub>2</sub> that in the

presence of water generates  $\text{HCO}_3^-$  lowering the pH (Sverdrup & Warfvinge 1995; Hoffland et al., 2004).

Mitchell et al., (2013) showed that subglacial bedrock mineralogy influences the microbial community inhabiting the subglacial environment. They showed that Fe-containing minerals supported the highest amount of microbial biomass, whereas silicates harboured the least. Nevertheless, silicates can contain some of the limiting elements for microbial growth such as feldspars, which contains P (Rogers and Bennett, 2004), or olivine, which contains Fe (Popa et al., 2012). To make these elements bioavailable, microorganisms can induce the dissolution of silicates altering the redox status of the metals entrapped in the mineral, drive production of excess protons, and using organic ligands (Rogers and Bennett, 2004). Interestingly, the presence of silicates in an aqueous medium at low temperature has been shown to produce  $\text{H}_2$ , which then can be used by microorganisms (Telling et al., 2015). Sequences belonging to taxa that harbour hydrogen-oxidisers were found in this study (e.g. *Comamonadaceae*, *Acidovorax*, *Polaromonas*), which could be using this molecule to obtain their energy.

Chemolithotrophy uses the oxidation of certain chemical species to obtain the energy necessary for the cell metabolism. Reduced and partially oxidised forms of nitrogen can be oxidised by several microbial genera. Chemical analyses of basal ice (Chapter 2) showed that N content in the sediment was as low as in the ice matrix ( $\sim 10^2$ ), but significant values of nitrate were found, being the most abundant form of nitrogen, suggesting that processes of N oxidation occur in basal ice. *Nitrosomonadaceae* is a phylogenetic group of chemolithoautotrophic nitrogen oxidisers, which includes the *Nitrosococcus* and *Nitrosospira*, both found in this work. *Nitrosococcus* and *Nitrosospira* are ammonia oxidising genera, oxidising  $\text{NH}_4^+$  to  $\text{NO}_2^-$  (Klotz et al., 2006).  $\text{NO}_2^-$  can then be oxidised by nitrite oxidisers. *Candidatus Nitrotoga* are nitrite oxidisers, transforming nitrite to nitrate at low temperatures (4-27°C) (Lücker et al., 2015). Members belonging to this group have been found in cold environments such as permafrost and in subglacial reservoirs, where it dominated the community in the water column (*Candidatus Nitrotoga arctica*) (Lücker et al., 2015; Mikucki et al., 2016;

Ishii et al., 2017). However, no statistically relevant correlation between the identified genera was found in this study.

S concentration was low in the sediment (~1 ppm), but sulphate levels were significant in the melted ice matrix (some cases >10 ppm), which suggests that reduced sulphur-bearing minerals can be oxidised in aqueous conditions in the subglacial environment, and the sulphate generated by this process is leached and accumulated in the veins. Sequences affiliated with S-oxidising genera were found in this work. *Thiobacillus* are strict chemolithotrophs that can oxidise H<sub>2</sub>S to S (Tóth et al., 2015), as well as inorganic sulphur compounds (i.e. thiosulphate, thiocyanate) in order to get their energy. Members of this genus have been described to be strict aerobes (*Thiobacillus thioparus*) or facultative anaerobes, using NO<sub>3</sub><sup>-</sup> as terminal electron acceptors (*Thiobacillus denitrificans*) (Hutt et al., 2017). *Thiobacillus denitrificans* also has the ability to oxidise certain metals, such as Fe and generates sulphate (Beller et al., 2006). Individuals belonging to this genus have been isolated and grown at low temperatures (Sattley and Madigan, 2006) and have been found in subglacial ecosystems dominating the microbial community in sediments. *Thiotricaceae* is a family included in the order *Thiotricales*, which includes chemolithotrophic S-oxidising genera, such as *Thiothrix*, *Thiomargarita*, *Thiobacterium*, *Thioploca*, *Beggiatoa*, and *Thiospira*. Members of this group are facultative aerobes, able to use nitrates as terminal electron acceptors accumulated in their vacuoles at high concentrations (Trubitsyn et al., 2013). *Acidiferrobacter* is a genus that comprises thermophilic acidophilic S-oxidisers, able to use pyrite, sulphides, sulphur or tetrathionate, but also reduced forms of Fe(II). They are facultative anaerobes able to use oxygen or Fe(III) as terminal electronic acceptors. Members of this genus are strictly chemolithotrophic and able to fix N<sub>2</sub> facultatively (Hallberg et al., 2011). *Gallionella* is a genus that includes mixotrophs able to grow using C sources, but also able to fix C (Hallbeck and Pedersen, 1991) using reduced sulphur compounds and Fe(II) and oxidising them in neutrophilic conditions (Lütters-Czekalla, 1990; Lanoil et al., 2009). Sequences affiliated with this genera have been found in subglacial environments, such as Antarctica soils and glaciers (Lanoil et al., 2009).

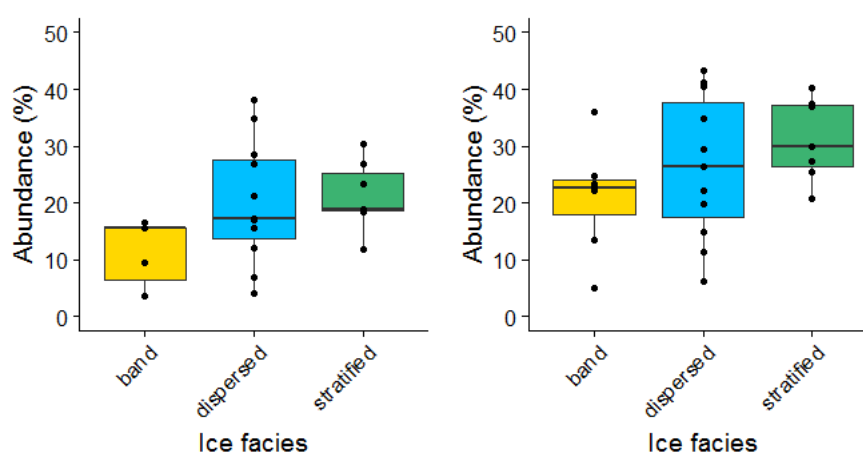
Fe was one of the most abundant elements detected by non-destructive in situ XRF analysis (Chapter 3, Section 3.3.1), and the most abundant metal in the sediment entrapped in basal ice (Chapter 3, Section 3.3.2), but the values in the ice matrix were very low (Chapter 3, Section 3.3.3), which suggests that Fe stays in an insoluble form in the basal ice environment, forming part of minerals, as was observed in the SPA analysis (Chapter 3, Section 3.3.4). Insoluble Fe(III) can be solubilised by direct or indirect reduction by genera such as *Geobacter*, *Ferrimicrobium*, *Rhodoferax* (Eisele and Gabby, 2014). Another way to solubilise Fe(III) is by the interaction with siderophores that can be produced by genera such as *Pseudomonas* (Eisele and Gabby, 2014), and has been described to help dissolve hematite, goethite, and ferrihydrite, among other iron-rich minerals (Dong, 2010). In acidic conditions, Fe becomes soluble, and therefore bioavailable for microbial metabolism. Fe(II) is another chemical species that can be used by chemolithotrophs in their metabolism. Sequences affiliated with Fe-oxidisers were identified, such as *Acidiferrobacter* and *Gallionella*, which are also S-oxidisers, as mentioned earlier. *Sideroxydans* members of this genus have been described to be aerobic Fe(II) oxidisers in neutrophilic (Sobolev and Roden, 2004) and acidophilic (Mühling et al., 2016) conditions fixing CO<sub>2</sub>. This group is closely affiliated with *Gallionella* (Sobolev and Roden, 2004). *Acidovorax* is a genus of the *Comamonadaceae* that comprises some chemolithoautotrophs, that are able to oxidise Fe(II) in anoxic conditions in the presence of NO<sub>3</sub><sup>-</sup> (Chakraborty et al., 2011). The activity of iron oxidisers can precipitate Fe(III) and Fe-hydroxides (Burford et al., 2003).

The presence of silicates in the subglacial environment can generate H<sub>2</sub> (Telling et al., 2015). Si was the most abundant element revealed by the XRF analysis, and silicates were the most abundant mineral in the SPA analysis, which would suggest that there is a supply of hydrogen in the basal ice at Svínafellsjökull. Sequences affiliated with H-oxidiser were identified, such as *Comamonadaceae*, which is a family that comprises heterotrophs and chemolithotrophs, which grow using H<sub>2</sub> (*Variovorax* and *Hydrogenophaga*), CO and Fe(II). They can be aerobes or anaerobes, using nitrate as terminal electron acceptor. Some members, such as *Hydrogenophaga* can fix nitrogen (Willems et al., 1991). Sequences belonging to *Comamonadaceae* have been

found in polar and alpine environments (Skidmore et al., 2005; Price, 2007; Cheng and Foght, 2007).

Stratified facies showed a statistically higher abundance of chemolithotroph-related sequences ( $32.7 \pm 6.4\%$ ) than debris bands ( $19.3 \pm 8.5\%$ ) and dispersed ( $17.0 \pm 6.4\%$ ) (Fig 4.8). *Thiobacillus*, *Thiotrichaceae*, *Gallionella* related sequences appeared in all stratified samples. This could mean that sulphur cycling is playing a very important role in stratified facies, considering that all these taxa are sulphur-oxidisers. Some minerals are formed by microbial activity, such as siderite, vivianite, magnetite, and green rust (Dong, 2010).

Sequences affiliated with microorganisms that have been implicated in mineral weathering were identified in this study. *Sphingomonas* species (that appears up to 1% in some of the samples) are able to weather biotite, phosphates and iron-containing minerals; *Acinetobacter* are able to weather phosphates; *Geobacter*, which can reduce Fe(III); *Thiobacillus*, is very well known for its ability to oxidise Fe(III) and use sulphate and *Pseudomonas*, which can weather phosphates and iron minerals (Uroz et al., 2009).



**Figure 4.8. Boxplot of the total percentage of sequences affiliated with chemolithotrophic species.** On the right, only including genera, and on the left, including families that are known to harbour chemolithotrophic species as well. Lines represent the median and the upper and lower limit of the box represent the 75th and 25th percentile respectively. Dots represent the outliers.

Fungal species are able to weather minerals (Hoffland et al., 2004), and fungal communities have been suggested to play very important roles in the formation of soil in recently deglaciated areas (Etienne, 2002). This is due to active fungal production of organic acids (particularly citric acid) that releases nutrients, especially

phosphorus. Low pH levels achieved in the fungal mycosphere can even result in the dissolution of silicates (Etienne and Dupont, 2002), which are the most abundant minerals in basal ice as analysed by SPA in this study. Basalts, such as the basalts that form the bedrock of Svínafellsjökull, have been found to be weathered by fungi in the foreland of glaciers in the southeast of Iceland (Etienne and Dupont, 2002),

#### 4.4.5 Relevance of basal ice microbiology to astrobiology

The results from this study suggest that the microbiota inhabiting basal ice will have a very important role in weathering at the interface between the glacier and the substrate. Microorganisms in basal ice will be interacting with the entrapped minerals, either oxidising them via chemolithotrophic metabolism, or reducing them using specific metals as terminal electron acceptors (Tranter et al., 2005). Subglacial environments, with their extreme characteristics (low temperature, oligotrophy, absence of light), but presence of viable and active microbiota, have been gaining attention within the astrobiological community (Skidmore et al., 2000; Tung et al., 2006; Buford Price, 2007; Fisher and Schulze-Makuch, 2013). Due to the presence of ice in different planets in our solar system, such as Mars or the icy moons of Saturn and Jupiter, terrestrial subglacial ecosystems and basal ice may provide analogues to understand the possibilities and constraints of life on these planets and moons.

The presence of a high abundance of sequences related to chemolithotrophic bacteria suggests that there is an input of carbon through fixation, sustaining an active ecosystem, which otherwise would not be viable based on the low C content found among basal ice samples. To maintain activity of the chemolithotrophic community, an input of fresh minerals would be necessary. Glaciers are known to be very effective at eroding their substrates, and the processes that generate basal ice at the ice-bed interface serve to entrain sediment. The main chemolithotrophic group found in this study are microorganisms that are known to interact with Fe and S containing minerals. Mars contains a high abundance of these two elements (Longhi et al., 1992; Achilles et al., 2017; Osterloo and Kierein-Young, 2017), making basal ice at Svínafellsjökull a good analogue for parts of this planet. In addition, the petrology of Mars is known to be dominated by basalts, which is the main bedrock beneath Svínafellsjökull.

#### 4.5 Conclusions

The microbial diversity inhabiting basal ice suggests that it is not the basal ice type that drives the microbial communities. However, some taxa showed statistically significant differences between the ice samples. Basal ice seems to be an active ecosystem, driven by the fixation of carbon by chemolithotrophs able to oxidise Fe – (*Gallionella*, *Acidiferrobacter*), S- (*Thiobacillus*, *Thiotricaceae*) containing minerals, as well as solutes dissolved in the available water surrounding grains and liquid brine veins, such as  $\text{NH}_4^+$  (*Nitrosomonas* and *Candidatus Nitrotoga*) and  $\text{NO}_2^-$  (*Nitrosococcus*). Heterotrophic communities will use the fixed C in their metabolisms and some members, such as *Lysobacter*, would have very important roles in the recycling of elements mediated by cell lysis. The capacities of the microbial communities to weather minerals and increase the C content can play a fundamental role in the colonisation of the glacier foreland, upon release from the ice matrix. Basal ice, and especially basal ice at Svínafellsjökull could represent a good analogue to understand the conditions of life in other planetary bodies, such as Mars or different icy moons of the gas giants in our solar system.



## 5. Quantification of basal ice microbial cell delivery to the glacier margin

### 5.1. Introduction

Glaciers and ice sheets are important ecosystems that support microbial life (Hodson et al., 2015). Although most work hitherto has focused on life at the glacier surface, the subglacial environment represents a potentially important but poorly understood microbial niche (Sharp et al., 1999; Doyle et al., 2013; Boyd et al., 2014; Montross et al., 2014). Debris-laden glacier basal ice plays an important role in sediment transfer to the ice margin because it is in contact with the glacier substrate and is involved in most of the geomorphological work achieved by a glacier (Knight, 1997; Hubbard et al., 2009). Whilst there is a body of research on the delivery of inorganic materials (i.e. bedrock and sediment) to the ice-marginal environment, and the associated development of landforms and sediments (Knight et al., 2002; Larson et al., 2006; Simon J Cook et al., 2011a), there is a dearth of information on the delivery of organic material, including microbes, to the glacier margin. An intriguing possibility is that microbes released from glaciers could play an important role in proglacial soil and vegetation development, yet despite a wealth of studies on vegetation succession in deglaciating environments (Nemergut et al., 2007; Shivaji et al., 2011; Zumsteg et al., 2012; Brown and Jumpponen, 2014), this has not yet been fully explored. Importantly, basal ice melt-out could deliver viable microbiota to the ice margin that serve as inoculum, potentially accelerating pedogenesis as glaciers recede (Kaštovská et al., 2007).

Unlike in the case of glacier surface ice, there remains limited literature quantifying viable or total microbial content of basal ice. Previous studies that have quantified the abundance of microorganisms inhabiting basal ice, suggest high variability in the numbers, but consistently report higher counts than snow-derived glacial ice (Table 4.1). Few studies have addressed the microbial diversity inhabiting basal ice. (Doyle et al., 2013) found a low diversity of microorganisms in the basal ice of Taylor glacier, dominated by *Firmicutes* and *Gammaproteobacteria*. Yde et al., (2010) found that the microbiology of the basal ice layer at the Greenland Ice Sheet was dominated by

*Gammaproteobacteria* and *Betaproteobacteria*, followed by *Bacteroidetes*. There is evidence that the basal ice constitutes an active environment demonstrated by the depletion of specific gases, such as O<sub>2</sub> forming anaerobic pockets (Wadham et al., 2007) and production of metabolism-related gases, such as CO<sub>2</sub> or CH<sub>4</sub> or nitrous oxide (Priscu, 1997; Sharp et al., 1999; Boyd et al., 2010; Doyle et al., 2013). Moreover, evidence of chemolithotrophy has been found, showing active utilisation of minerals, such as pyrite (Mitchell et al., 2013). Taken together, these findings support a hypothesis identifying basal ice as a niche and eventual source of high microbial numbers to the glacier forefront.

**Table 5.1. Direct cells counts in basal ice previously reported in the literature**

Glacier	Cell counts (cells g <sup>-1</sup> )	Source
<b>Glacier de Tsanfleuron</b>	10 <sup>7</sup>	(Sharp et al., 1999)
<b>Greenland Ice Sheet</b>	10 <sup>11</sup>	(Tung et al., 2006)
<b>Greenland Ice Sheet</b>	2 x 10 <sup>8</sup>	(Yde et al., 2010)
<b>Taylor glacier</b>	2.6 - 4.9 x 10 <sup>2</sup>	(Doyle et al., 2013)
<b>Taylor glacier</b>	1.8 x 10 <sup>3</sup> - 1.8 x 10 <sup>4</sup>	(Doyle et al., 2013)
<b>Taylor glacier</b>	10 <sup>2</sup> - 10 <sup>4</sup>	(Montross et al., 2014)
<b>Engabreen glacier</b>	6.8 x 10 <sup>5</sup>	(Lawson et al., 2015)
<b>Finsterwalderbreen glacier</b>	1.7 x 10 <sup>5</sup>	(Lawson et al., 2015)
<b>Russell glacier</b>	2.3 x 10 <sup>5</sup>	(Lawson et al., 2015)
<b>Joyce glacier</b>	1.2 x 10 <sup>5</sup>	(Lawson et al., 2015)

Few studies have quantified sediment discharge from basal ice due to incomplete knowledge of subglacial systems (Wainwright et al., 2015), although this has been achieved at a few well-studied sites (Hunter et al., 1996; Knight et al., 2002; Larson et al., 2006; Cook et al., 2010; Cook et al., 2011b). Debris flux depends on the glacier with values of 12.3-44.8 m<sup>3</sup> m<sup>-1</sup> a<sup>-1</sup> for Russell glacier (Knight et al., 2002), 56 m<sup>3</sup> m<sup>-1</sup> a<sup>-1</sup> for Matanuska glacier (Larson et al., 2006). Hunter et al., (1996) used different calculation procedure obtaining values ranging between 9.7 x 10<sup>5</sup> and 1.3 x 10<sup>6</sup> m<sup>3</sup> a<sup>-1</sup> for Alaskan tidewater glaciers. It has been discussed that basal ice, due to transporting most of the sediment would be the limiting factor for moraine growth (Knight et al., 2002).

To our knowledge, no studies have yet addressed both sediment transport and microbial content simultaneously. Our aims were to determine cell discharge from

debris-bearing basal ice, and confirm that cultivable microbial inoculum is transferred between glaciers and proglacial ecosystems through the basal ice layer. This study was undertaken at Svínafellsjökull, Iceland, which is one of only a few well described glaciers with regards to the physical characteristics of basal ice and its formation and sediment discharge to the ice margin from individual basal ice facies (Cook et al. 2007, 2010, 2011a,b).

## 5.2. Study site and methods

### 5.2.1 Svínafellsjökull

Svínafellsjökull (63°59' N, 16°52' W) is a temperate outlet glacier of the Öræfajökull ice cap, southeast Iceland (Figure 1). It descends from the ice cap summit down an icefall and terminates in a subglacial overdeepening with a total distance of approximately 7 km. Historical records indicate that between 1350 and 1500, the terminus was approximately 3.5 km further up-valley than it is today, with part of the area currently covered by the glacier used for agriculture (Ives, 2007). This advance-recession pattern has been followed by a number of glaciers in this region (Hannesdóttir et al., 2015). In the last 10 years, some water bodies in the foreland of Svínafellsjökull have developed, but a great part of the glacier margin is still in contact with moraine/till. It is likely, therefore, that Svínafellsjökull has overridden ancient soil and vegetation, which may now constitute part of the subglacial debris load. Ives, 2007 highlights documentary reports from neighbouring Skaftafellsjökull where the glacier had once been mined for birch wood. A similar situation has been described in Svalbard, where relict vegetation with a microbiota associated was described underneath Longyearbreen glacier (Humlum et al., 2005).

### 5.2.2 Basal ice samples

Basal ice is typically heterogeneous in physico-chemical composition (Knight, 1997; Hubbard et al., 2009) and could thus be hypothesised to exhibit spatio-temporal variation in numbers and diversity. Unfortunately, previous studies of subglacial microbiology have not always provided adequate descriptions of the ice and sediment being sampled. Basal ice at Svínafellsjökull has been described in detail by Cook et al. (2007, 2010, 2011b) and, broadly, comprises two main facies. Firstly, 'stratified facies' that is characterised by high sediment content (approximately 32 %

by volume) displays a distinctive layered appearance and is localised as a layer up to 4 m thick in contact with the glacier bed around the southernmost part of the glacier margin. Cook et al. (2007, 2010) demonstrated that much of this facies was formed through glaciohydraulic supercooling in the terminal overdeepening. Cook et al. (2007, 2010) also described a second population of stratified facies formed by regelation, but this was not observed in the present study. Secondly, 'dispersed facies' (Cook et al., 2011b) is characterised by lower sediment content (1.6 % by volume), but is found pervasively around the glacier margin in a layer up to 25 m thick, either in contact with the glacier bed, or overlying the stratified facies. Cook et al. (2011b) suggested that dispersed facies was formed by tectonic entrainment of sediment at the base of the icefall, followed by regelation, deformation and strain-related metamorphism.

Three stratified and three dispersed facies samples were collected aseptically from Svínafellsjökull in April 2015 following methods outlined in Toubes-Rodrigo et al. (2016) (sample locations on Chapter 2). Briefly, the glacier margin was surveyed in order to find accessible basal ice facies (Figure 4.1). Although some water bodies have developed over the last years, most of the basal ice is still in contact with till and/or moraine, as can be observed. Then, using an ice axe, the first 20-30 cm of the basal ice facies were removed. Ice blocks were carved using flame-sterile chisels and carefully deposited in double or triple sterile plastic bags, depending on the ice blocks sharpness, to minimise the potential of bag piercing and cross-contamination. The distribution, type and thickness of basal ice was similar to that described by Cook et al. (2007, 2010, 2011b). Ice samples were allowed to melt over 2 weeks at 4°C and sediment-bearing water was poured into 50 ml sterile Falcon tubes. To preserve the cells, formaldehyde was added to a final 4% v/v concentration. Samples were kept at 4°C until further analysis.

### 5.2.3 Cell counts

Fixed samples were sonicated for 3 minutes using an S-Series Table Top ultrasonicator (Sonicor Inc. USA) at maximum intensity to release the cells from the sediment grains, and 1 ml was extracted from the supernatant and filtered using 0.2 µm Whatman® Nuclepore™ Track-Etched Membranes. For cell enumeration in

sediment entrained in basal ice samples, dried filters were stained using 4'-6-diamidino-2-phenylindole (DAPI) ( $1 \text{ mg ml}^{-1}$ ) and covered with Leica type P immersion liquid prior to examination in an epifluorescence microscope Nikon Eclipse E600 (Nikon, Japan) (Lunau et al., 2005)

#### 5.2.4 Colony forming units (CFU) count in sediment

To determine if the community inhabiting the basal ice was alive and cultivable, 1 g sub-sample was diluted in 9 ml of saline buffer (0.85 % w/v NaCl) and vortexed for 2 minutes at full speed (Clifton Cyclone MIX5050 vortexer, SLS, UK). Serial dilutions were performed in the saline buffer from  $10^{-1}$  to  $10^{-5}$ , and transferred for isolation onto 1:10 Tryptone Soy Agar (TSA) plates with cycloheximide (100 mg/ml) (Elliott et al., 2015) a standard general non-specific medium to compare differences in the abundance cultivable cells between ice facies. Plates were incubated at  $4^{\circ}\text{C}$  for 5 weeks and colonies were counted.

#### 5.2.5 Cell discharge calculations

Svínafellsjökull is one of the few glaciers where basal ice sediment transfer has been quantified. Cook et al. (2010) calculated that sediment flux through the stratified facies was  $4.8$  to  $9.6 \text{ m}^3 \text{ m}^{-1} \text{ a}^{-1}$  (i.e. the volume of sediment transferred per metre of basal ice exposure around the glacier margin, per year), with the range in values determined by the range in previously measured glacier velocities. The equivalent value for sediment discharge was calculated as  $207$  to  $414 \text{ m}^3 \text{ a}^{-1}$  (Cook et al., 2011b). These values relate specifically to the stratified basal ice formed by glaciohydraulic supercooling. For the current study, stratified basal ice associated with regelation was not observed, as it was by Cook et al. (2010), and so is not considered here. Cook et al. (2010, 2011b) calculated a sediment flux of  $1.0$  to  $2.0 \text{ m}^3 \text{ m}^{-1} \text{ a}^{-1}$ , and a sediment discharge of  $42$  to  $84 \text{ m}^3 \text{ a}^{-1}$ , for this so-called 'non-supercool' basal ice, so its contribution to cell discharge would have been much lower even if it were present. Cook et al. (2011b) calculated that sediment flux through the dispersed facies was  $0.81$  to  $1.62 \text{ m}^3 \text{ m}^{-1} \text{ a}^{-1}$ , and that sediment discharge was  $1635$  to  $3270 \text{ m}^3 \text{ a}^{-1}$ . The very high sediment discharge relative to stratified facies is because dispersed ice is found ubiquitously around the glacier margin in metres-thick sequences, despite its low sediment content.

We follow glacial sediment transfer convention by quantifying analogous cell transfer values as ‘cell discharge’, in cells  $\text{m}^{-1} \text{a}^{-1}$ , and ‘cell flux’, in cells  $\text{m}^{-1} \text{a}^{-1}$ . To calculate these, cell concentration, expressed as the number of cells per kilogram of sediment (cells  $\text{kg}^{-1}$ ), was multiplied by the sediment discharge ( $\text{m}^3 \text{a}^{-1}$ ) or flux ( $\text{m}^3 \text{m}^{-1} \text{a}^{-1}$ ), and then multiplied by sediment density ( $\text{kg m}^{-3}$ ). The geology of the study region is dominated by basalt ( $\rho_{\text{basalt}} = 2800\text{-}3000 \text{ kg m}^{-3}$ ) and hyaloclastite ( $\rho_{\text{hyaloclastite}} = 2750 \text{ kg m}^{-3}$ ) (Magnússon et al., 2012). For the purpose of this work, we assume a sediment density ( $\rho_{\text{sediment}}$ ) of  $2800 \text{ kg m}^{-3}$ .

### 5.3. Results

#### 5.3.1 Cell discharge from basal ice

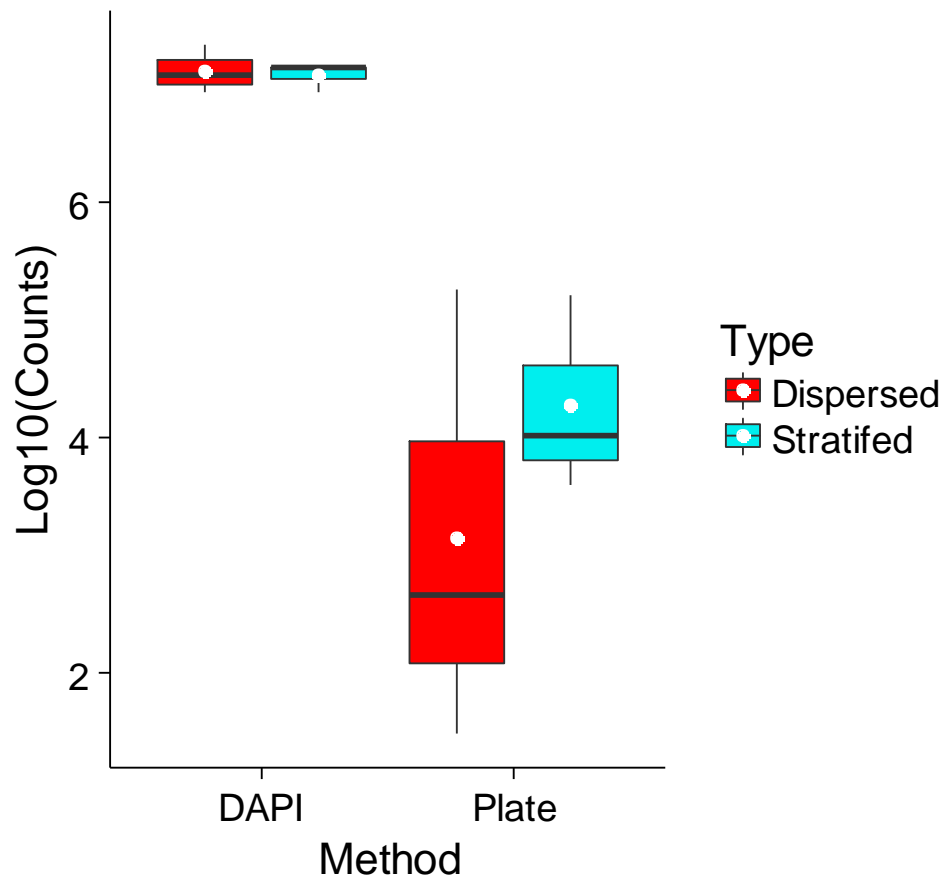
Cell counts reveal that stratified facies contains  $1.3 \times 10^7$  cells  $\text{g}^{-1}$  of sediment ( $n = 3$ ;  $\sigma = 2.7 \times 10^6$ ), whilst dispersed facies contains  $1.4 \times 10^7$  cells  $\text{g}^{-1}$  of sediment ( $n = 3$ ;  $\sigma = 5.7 \times 10^6$ ) (Fig 5.1). Hence, stratified and dispersed facies have similar cell counts and no statistically significant differences were observed (t-test p-value = 0.72). Cell counts in supernatant (data not shown) were insignificant compared with sediment.

Cell discharge associated with the sediment within stratified facies is between  $7.4 \times 10^{15}$  to  $1.5 \times 10^{16}$  cells  $\text{a}^{-1}$ , and is between  $6.6 \times 10^{16}$  to  $1.3 \times 10^{17}$  cells  $\text{a}^{-1}$  for dispersed facies. Hence, 10.1% of cell discharge per year is associated with stratified facies, and 89.9% is associated with dispersed facies. The cell flux through stratified facies is between  $1.8 \times 10^{14}$  and  $3.5 \times 10^{14}$  cells  $\text{m}^{-1} \text{a}^{-1}$ , and for dispersed facies this value is between  $3.3 \times 10^{13}$  and  $6.6 \times 10^{13}$  cells  $\text{m}^{-1} \text{a}^{-1}$ . Hence, where stratified facies is present, cell flux is 5.4 times more cells than dispersed facies, but because of a more limited distribution around the ice margin, overall cell discharge is much lower than dispersed facies.

#### 5.3.2 Enumeration of cultivable cells

For both stratified and dispersed facies, CFU values were similar without statistically significant differences between them (t-test p-value = 0.43) and overlap can be observed in the box and whiskers plot in Figure 5.1. Nevertheless, dispersed basal ice counts were more variable ( $n = 3$ ,  $\bar{x}_{\text{dispersed}} = 5.9 \times 10^4$ ,  $\sigma^2_{\text{dispersed}} = 1.2 \times 10^{10}$ ) with three orders of magnitude difference between samples D1 and D3, whereas counts from

stratified facies ( $n = 3$ ,  $\bar{x}_{\text{stratified}} = 5.9 \times 10^4$ ,  $\sigma^2_{\text{stratified}} = 7.9 \times 10^9$ ) were more consistent, with less than an order of magnitude of difference between samples.



**Figure 5.1.** Total cell counts using DAPI and CFU counts of 1:10 TSA plates after 5 weeks incubation for the two types of ice, stratified (in blue) and dispersed (in red) ( $n=3$  for both ice facies). The box plots show the smallest and largest values, 25% and 75% quartiles, and the median. Points represent the mean of the  $\text{Log}_{10}(\text{CFU/g})$ .

## 5.4. Discussion

### 5.4.1 Determination of total and cultivable cell content in basal ice

We have demonstrated that there are large numbers of cells ( $\bar{x}_{\text{stratified}} = 1.3 \times 10^7$  cells  $\text{g}^{-1}$ ,  $\bar{x}_{\text{dispersed}} = 1.4 \times 10^7$  cells  $\text{g}^{-1}$ ) associated with sediment trapped in basal ice at Svínafellsjökull, both for the stratified and dispersed facies. For comparison, Lawson et al. (2015) quantified cell counts in the basal ice of four different glaciers comprising examples of polythermal, warm- and cold-based glaciers, and found lower cell counts, on the order of  $10^5$  cells  $\text{g}^{-1}$ ; the highest values were found in the warm-based glacier, Engabreen, whereas the lowest values were found in the cold-based Joyce Glacier. One possible explanation for the high cell counts at Svínafellsjökull is that this

glacier likely overrode soils and vegetation, and associated microbiota, during its Little Ice Age advance (Ives, 2007). Nonetheless, cell numbers within basal ice are much lower than in proglacial soil, where typical cell numbers would be on the order of  $10^8$  cells  $\text{g}^{-1}$  (Shivaji et al., 2011).

Successful cultivation of microorganisms in 1:10 TSA plates at  $4^\circ\text{C}$  demonstrates that microbiota present within basal ice are viable and cultivable. These microorganisms, once released to the ice-marginal environment, would be able to proliferate and assist in the initiation of soil formation as the glacier recedes (Brown and Jumpponen, 2014; Rime et al., 2016). It is noteworthy that only between 1-3% of the total microbiota can be isolated using traditional methods (Armougom and Raoult, 2009), so although only up to  $10^5$  bacterial cells per gram of sediment were observed to have grown, the actual number of viable cells is likely to be up to 100-fold higher.

#### 5.4.2 Quantification of cell discharge through the basal ice layer

For the first time, we have quantified microbial cell transfer to a glacier margin through the basal ice layer. This value depends on glacier velocity, which was calculated either by *in situ* measurements (Cook et al., 2010) and by remote sensing (*unpublished S. Cook*) to be of  $6 \text{ cm day}^{-1}$ , ranging between  $4$  and  $8 \text{ cm day}^{-1}$  (or  $14.6$  to  $29.2 \text{ m a}^{-1}$ ). For stratified facies, the cell flux is between  $1.8 \times 10^{14}$  and  $3.5 \times 10^{14}$  cells  $\text{m}^{-1} \text{ a}^{-1}$ , which corresponds to a total discharge of  $7.4 \times 10^{15}$  to  $1.5 \times 10^{16}$  cells  $\text{a}^{-1}$ . For dispersed facies, the cell flux is between  $3.3 \times 10^{13}$  and  $6.6 \times 10^{13}$  cells  $\text{m}^{-1} \text{ a}^{-1}$ , which corresponds to a cell discharge of  $6.6 \times 10^{16}$  to  $1.3 \times 10^{17}$  cells  $\text{a}^{-1}$ . The cell flux is one order of magnitude higher in stratified facies than in dispersed facies. However, the overall cell discharge from stratified ice is much lower due to the far greater thickness and abundance of dispersed facies around the glacier margin. It is clear, that different ice facies deliver different amounts of cells to the glacier margin, indicating that future studies of basal ice microbiology should account for ice facies characteristics, as has been the norm for glaciological studies of basal ice (e.g. Hubbard et al., 2009). Previous studies have shown very variable cells numbers in basal ice (Table 5.1). Our results are the most similar to the ones obtained at Tsanfleuron glacier, another temperature valley glacier, but in a different location, in the Alps.



## 5.5. Conclusions

Using a combination of glaciological and microbiological techniques we have estimated the delivery of microbial cells through the basal ice later. Our results suggest that there is an abundant microbial community surrounding sediment grains within the subglacial environment, which delivers large amounts of dead and viable cells to the ice margin at Svínafellsjökull. Dead cells represent a potentially important nutrient resource to the proglacial environment, which may promote colonisation by pioneering communities. These pioneering communities can have been entrained from overridden soil during glacier advance (Skidmore et al., 2000; Humlum et al., 2005; Ives, 2007) and could be delivered by deposition and melt-out, making the first steps of the pedogenesis independent from the external input. Although microorganisms were able to be grown on 1:10 TSA, therefore confirming the presence of viable bacterial communities within the ice. However, only one condition was used to analyse the presence of culturable microbiota, which is likely to represent a minor proportion of total viable community. Hence, our results represent conservative estimates of the delivery of viable cells to the ice margin.

Our results represent an individual case study based on one temperate Icelandic glacier. As demonstrated by Lawson et al. (2015), cell numbers in basal ice vary greatly according to glacier thermal regime, and other factors, such as substrate type (e.g. sediment vs. bedrock) and lithology, may also play important roles in determining the nature and size of subglacial microbial communities. Even within our dispersed facies samples, there is variability in CFU counts between samples (Figure 2). Hence, we recommend that similar studies be performed at other sites with different glaciological characteristics to gain a better appreciation of cell transfer to the margins of glaciers.

## 6. Cultivable bacterial microbiota of Svínafellsjökull: Importance in soil development.

### 6.1 Introduction

The basal ice layer of glaciers and ice sheets is dark, oligotrophic, and cold. However, it harbours rich and active microbiota (Yde et al., 2010; Bakermans and Skidmore, 2011; Montross et al., 2014), strongly represented by chemolithotrophic communities (Boyd et al., 2014). In order to thrive, these individuals can oxidise the minerals entrapped in the basal ice, obtaining energy from this reaction, and fixing carbon (Tung et al., 2006). Consequently, minerals are weathered and enriched in C. Additionally, heterotrophic communities can weather minerals by a range of processes (e.g. acid production, siderophore production (Uroz et al., 2009), in order to mobilise the constituent nutrients. Yet, information and knowledge of the abundance, diversity, and relevance of the basal ice microbiota remains poorly understood (Toubes-Rodrigo et al., 2016). At their termini, glaciers release the debris entrained in the basal ice (Knight et al., 2002; Cook et al., 2006; Cook et al., 2011a), and this sediment contributes to the foreland and frontal moraine systems, leading to the formation of new terrestrial ecosystems (Kaštovská et al., 2005; Brown and Jumpponen, 2014; Rime et al., 2016). Microorganisms released from the glacier can act as a primary inoculum and trigger the process of soil formation by weathering rock minerals and contributing organic matter (Rime et al., 2016). The resulting exposed glacier substrate and bedrock chronosequence has attracted considerable attention with regards to their evolution into fully developed soil, which can support vegetation establishment and succession (Zumsteg et al., 2012; Brown and Jumpponen, 2014; Schmidt et al., 2014; Kim et al., 2017). Recently deglaciated soils are exposed to high stress conditions, such as freeze-thaw cycles and desiccation, but still support significant microbial communities (Bradley et al., 2016; Rime et al., 2016). In addition, the soils close to the glacier margin tend to be vegetation free and limited in C and N as well as available nutrients (Haeberli et al., 2007).

Until recently, it was believed that pioneering microbial community inocula in deglaciating forelands were derived from deposition from exogenous sources, and

transported by means such as wind or precipitation (Womack et al., 2010; Chuvochina et al., 2011; Temkiv et al., 2012). However, recent work by Rime et al. (2016) compared the different communities inhabiting snow, basal ice, and periglacial ecosystems and concluded that basal ice plays a very important role in the delivery of viable cells to glacier forelands. The cultivable microbiota from cold environments has been shown to be dominated by psychrotolerant microbes, such as *Polaromonas*, *Cryobacterium*, *Flavobacterium*, and other microorganisms that can proliferate at low temperatures (Skidmore et al., 2005; Shivaji et al., 2011). Microorganisms entrapped in ice have the potential for viability and thus productivity. For example, in dark environments, chemolithotrophic bacteria have been identified that obtain energy while oxidising minerals and fixing C, such as at sea floor hydrothermal vents (Reysenbach et al., 2000; Nakamura and Takai, 2014) or subsurface ecosystems (Amend and Teske, 2005; Momper et al., 2017). The microbial activity in basal ice would make the sediment suitable for subsequent colonisation (Boyd et al., 2014).

Microorganisms in subglacial environments have been described to play very important roles in biogeochemical cycles, such as N (Yde et al., 2010; Boyd et al., 2011), Fe (Christner et al., 2014; Nixon et al., 2017), S (Mitchell et al. 2013; Boyd et al. 2011), and C (Stibal, Hasan, et al., 2012; Stibal, Wadham, et al., 2012; Boyd et al., 2014). C in the subglacial environment can appear as organic carbon (OC), which can fertilise the proglacial environment upon release (Lawson et al., 2015), or as gases, such as CO<sub>2</sub> and CH<sub>4</sub> (Montross et al., 2014). If the ice matrix surrounding these gases disappears, they can escape to the atmosphere, and some of the gases that have been identified in subglacial environments are greenhouse gases, such as CO<sub>2</sub> (Montross et al., 2014) and CH<sub>4</sub> (Wadham et al., 2012).

The microorganisms involved in primary colonisation of glacier forelands include: bacteria, especially non-autotrophic chemolithotrophs, which can weather minerals via redox reactions and can fix CO<sub>2</sub>, thereby increasing organic C content (Brankatschk et al., 2011); cyanobacteria and algae, which increase organic C content by photosynthetic activity (Kaštovská et al., 2007; Zumsteg et al., 2012) and heterotrophic bacteria and fungi (in many cases forming lichens associated with

phototrophs), which can release nutrients via the production of organic acids, siderophores, and extracellular polysaccharides (Frey et al., 2010). As a result, proglacial sediments become incorporated into cryptogamic soil characterised by higher surface cell content, higher organic C content and more weathered minerals (Zumsteg et al., 2012). In addition, N-fixing bacteria can increase the organic N-content of the sediment, which overcomes N limitation - a significant factor for higher plant establishment in soils (Brankatschk et al., 2011). The subsequent plant community development is dependent on primary colonisation and succession, led by microorganisms. Several studies have shown shifts in the microbial communities inhabiting proglacial soils of different ages (Kaštovská et al., 2005; Zumsteg et al., 2012; Brown and Jumpponen, 2014; Schmidt et al., 2014; Kim et al., 2017).

Since the advent of massive sequencing techniques and metagenomics and their application in environmental microbiology, it has been obvious that only a very small fraction of the total microbiota is cultivable on standard microbiological media (Vartoukian et al., 2010; Pham and Kim, 2012; Overmann et al., 2017). The low representation of the cultivable microbiota means that the information provided by cultivation alone is very biased. Nevertheless, the cultivation of microorganisms is crucial in order to characterise the metabolism of such microorganisms (Pham and Kim, 2012). In order to resolve issues on low cultivability, Nichols et al., (2010) developed isolation chips, or simply ichips. In summary, ichips comprise an array of microperforated wells, which can hold 1 µl of agar. Dilution from environmental samples is then performed in order to have a single cell in each well. Both sides of the microperforated sheet are then covered with two 0.22 µm filters. This allows the diffusion of nutrients, emulating the natural conditions in which the microorganisms exist. Finally, the filters are covered with another set of microperforated sheets and are tightened in order to avoid any contamination inside of the wells. The ichip has contributed to increases in the cultivable diversity by up to 50% in temperate soils, and to the discovery of novel species and metabolites (Lincke et al., 2010; Hames-Kocabas et al., 2012; Ling et al., 2015).

The importance of cold-adapted microorganisms has been highlighted in recent years, due to their ability to produce enzymes adapted to low temperatures, which

have potential applications in industry. (Gesheva, 2010; Jurelevicius et al., 2012; Bajaj and Singh, 2015; Lamilla et al., 2016). Cold-adapted enzymes present a range of characteristics that allow them to remain active at low temperatures, and they present a high catalytic constant ( $k_{cat}$ ). In addition, they can be easily denatured at high temperatures, allowing a simple control of the enzymatic activity (Cavicchioli et al., 2011). Industries that use cold-adapted enzymes include food industries (lipases – improvement of digestibility, flavour modification,  $\beta$ -galactosidase – dough fermentation), environmental biotechnology (lipases – bioremediation, biodiesel production), and the textile industry (amylases – denim desizing) (Cavicchioli et al., 2011).

This chapter focuses on developing a greater understanding of the links between the microbiology of the basal ice layer, and the proglacial zone where that material is released through melt-out at the glacier terminus. The main aims of this work, which targets sediment, basal ice, and forefield soil at Svínafellsjökull are: (1) To determine cultivable bacteria abundance and diversity, (2) to analyse cultured microorganisms from proglacial soil and sediment for activity at psychrophilic conditions, and (3) identify the potential origin and fate of the microorganisms entrapped in basal ice.

## 6.2 Material and methods

### 6.2.1 Site Descriptions and Sampling

Three geo-microbiological sampling campaigns were carried out at Svínafellsjökull, southeast Iceland. In August 2012, a general sampling transect was established that encompassed the glacier itself through to the outer moraine. This allowed the sampling of supraglacial sediment (SUP), subglacial sediment, which included samples of subglacial till (ST) and melt-out till (MOT), and soil, which included young soil sampled from the up- and down-glacier faces of a seasonal push moraine (IPM – inner face of the push moraine; SPM – outer face of the push moraine), and old soil (Little Ice Age moraine – LIA; Storalda moraine - STOR). All sample locations are shown in Chapter 2, Figure 2.1.

The Storalda moraine is the outermost moraine visible in the forefront of Svínafellsjökull. The uppermost 40 cm of this moraine, where the sample was

extracted, has been reported to contain discrete tephra layers (Gonzalez et al., 1999). The Little Ice Age (LIA) moraine corresponds to the position where the glacier terminus was located during the Little Ice Age (~1890) and is a prominent system of moraines situated ~250 m from the glacier margin (Hannesdóttir et al., 2015). Seasonal push moraines (samples SPM and IPM) were formed by seasonal glacier advance during the winter of 2011/12 where ice pushed into proglacial sediment to build debris mounds of approximately 3 m in height. Subglacial till (ST) represents sediment found in contact with the glacier base, and which may be formed by a range of processes including melt-out, lodgement and deformation (Evans et al., 2006). MOT is a type of subglacial till that was defined by Evans et al., (2006) as, “sediment released by melting or sublimation of stagnant or slowly moving debris-rich glacier, and directly deposited without subsequent transport or deformation”. It has been suggested that MOT may inherit the sedimentological characteristics of its parent basal ice (Cook et al., 2011a). SUP was sampled from cryoconite holes on the glacier surface ~250 m from the glacier margin, and corresponds to sediment whose origin may be the rock walls surrounding the glacier, or dust fragments that have been swept-in by the wind.

For supraglacial debris, till and moraine samples, samples were directly pushed into 30 ml plastic universal tubes and stored on ice for transport, and then stored in a refrigerator at 4°C until further processing.

Stratified and dispersed facies basal ice and debris bands were sampled under aseptic conditions from Svínafellsjökull in April 2015 and May 2016, following the methodology outlined in Toubes-Rodrigo et al. (2016). Sample locations are indicated in Chapter 2, Figure 1. Briefly, the glacier margin was surveyed to find accessible basal ice facies representing stratified and dispersed facies, and debris bands (Chapter 2). Although some proglacial water bodies have developed over recent years, basal ice remains in contact with proglacial till and/or moraine in a number of locations. Using an ice axe, the first 20-30 cm of the basal ice or debris band was removed. Ice blocks were carved out using flame-sterile chisels and carefully deposited in double or triple sterile plastic bags, depending on the ice block edge configuration and sharpness, to minimise the potential of bag piercing and cross-contamination. The distribution,

type and thickness of basal ice was similar to that described by Cook et al., (2007, 2010, 2011a, b). Ice samples were allowed to thaw over 48 hours at 4°C and sediment-bearing meltwater was decanted into 50 ml sterile Falcon tubes.

#### 6.2.2 Microbial isolation and enumeration via classic dilution plating

A 1 g sub-sample of each sample was diluted in 9 ml saline buffer (0.85 % w/v NaCl) and vortexed for 2 minutes at full speed (Clifton Cyclone MIX5050 vortexer, SLS, UK). Serial dilutions were performed in saline buffer from  $10^{-1}$  up to  $10^{-5}$ . For isolation, 1:10 Tryptone Soy Agar (TSA) plates (pH 7.3) (Difco Microbiology, UK) with cycloheximide (100 mg/ml) (Sigma-Aldrich, UK) (Bulluck III and Ristaino, 2002) were inoculated and incubated at 10°C for 18 months in light-proof sterilized containers. Colonies were counted. Based on morphological criteria, different colony types were picked after this incubation and streak-plated to fresh plates of 1:10 TSA + cycloheximide. These plates were poured with autoclaved and cooled agar amended with Tryptone Soy Broth (TSB) (Sigma-Aldrich, UK) that were autoclaved separately to minimize the formation of inhibitory peroxides, following Tanaka et al. (2014). This streak plating was repeated until pure cultures were observed. The pure strains were stored at -80°C in 1:10 TSA supplemented with 30% (v/v) glycerol.

For the isolation of iron oxidisers, a ferrous sulphate-containing media (FSM) was prepared (Linton, 2003). Prior to preparing the media, three solutions need to be prepared:

- 1) Solution 1: 200 ml dH<sub>2</sub>O and 8 g of agarose and autoclaved for 15min at 121°C
- 2) Solution 2: 100 ml of Basal Salt Solution, 0.5 ml of Trace Elements Solution, Sea Salts (0, 15, 30 g) and pH needs to be adjusted to 2.5 using H<sub>2</sub>SO<sub>4</sub> and autoclaved for 15min at 121°C
- 3) Solution 3: 50ml dH<sub>2</sub>O and 1g of FeSO<sub>4</sub> and pH needs to be adjusted to 2.5. In order to diminish the risk of air-induced oxidations, this solution needs to be prepared just before media preparation.

The compositions of the Basal Salt Solution and Trace Element Solution are the following:

- a) **Basal salt solution:** 1.5 g of  $(\text{NH}_4)\text{SO}_4$ , 0.5 g of KCl, 5 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 0.5 g of  $\text{KH}_2\text{PO}_4$  in per litre of distilled water ( $\text{dH}_2\text{O}$ ). The pH of the solution needs to be adjusted to 2.5 using  $\text{H}_2\text{SO}_4$ . This solution is autoclaved at  $121^\circ\text{C}$  during 15 mins and stored at  $4^\circ\text{C}$  until use.
- b) **Trace elements solution:** 10 g of  $\text{ZnSO}_4$ , 1 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 1 g of  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 1 g of  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g of  $\text{Cr}_2(\text{SO}_4)_3 \cdot 15\text{H}_2\text{O}$ , 0.5 g of  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ , 0.5 g  $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ , 0.1 g of  $\text{NaVO}_3$ . In order to solubilise the salts, gently mixing by rolling the bottle is required, and once dissolved sterilised by filtering and stored at  $4^\circ\text{C}$  until use.

For isolation of Fe oxidisers, 1 g of basal ice sediment was mixed with 9ml of sterile water and transferred onto plates and spread thoroughly and incubated at  $4^\circ\text{C}$ , until growth was observed, until a period of 1 year had elapsed.

Growth of bacteria in liquid medium isolated from the target sediment samples from Svínafellsjökull was monitored at incubation temperatures of  $4^\circ\text{C}$ ,  $25^\circ\text{C}$  and  $37^\circ\text{C}$ , as well as a range of NaCl concentrations (0%, 0.5%, 2% and 5%) at  $4^\circ\text{C}$ . Isolated bacteria were cultivated at the indicated temperature and salt conditions in 96 well-plates containing 1:10 Tryptone Soy Broth (1:10 TSB) with added aliquots of each inoculum, and the growth was monitored every 24 hours until a stationary phase was reached (268 hours for the temperature analysis and 408 for the salt concentration analysis). Optical density at 578 nm ( $\text{OD}_{578}$ ) was measured using an automated plate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Fisher Scientific, USA).

#### 6.2.3 Microbial isolation and enumeration via isolation chips (ichips)

Isolation chips were used for basal ice samples from the 2015 and 2016 campaigns. The ichip (Nichols et al. 2010) fabricated for this study was modified as follows: a circular central piece made of Delrin® microperforated sheet, two outer microperforated polycarbonate discs and two outer 3D printed clamp rings. Culture wells of 1 mm diameter had been cut (1  $\mu\text{l}$ ) in the central Delrin® sheet and the outer 3D-printed clamping rings allowed increased pressure application to avoid leakage and cross-contamination. Sediment was diluted  $10^3$ ,  $10^4$ ,  $10^5$  times in sterile distilled water and thoroughly mixed with melted Phytigel (final concentration 10 g/L) (Sigma Aldrich, now Merck, UK) in sterile bags under aseptic conditions. Phytigel was chosen



due to its low melting point, minimising damage to the cold-adapted cells. The sterile Delrin® culture sheet was added to each bag and phytagel-inoculum dilution massaged to ensure all wells contained phytagel. Ichips were assembled under sterile conditions, with outer sterile polycarbonate membranes (Whatman, UK, pore size 0.22µm, 47 mm diameter) and transferred to sterile bags containing basal ice, and maintained sealed at 0-4°C for 10 days. After this period the ichips were aseptically disassembled and membranes were transferred to the surface of 1:10 TSA plates and incubated for 5 weeks at 4°C, tracking the appearance of colonies and being transferred to fresh 1:10 TSA for culture purification. This was done because the agar plug disappeared from the micro-perforations.



**Figure 6.1. Circular ichip incubated in dispersed facies samples inside of sterile bags.** In the picture, 3D printed black clamping rings to maintain the structure can be observed, and the three layers of last perforated plastic. The glove was used to keep the label intact inside and isolated from water.

#### 6.2.4 16S rDNA amplification and phylogenetic analysis

Identification of the isolates was carried out by partial sequencing of the phylogenetic marker 16S rRNA gene (Doyle et al., 2013). The 16S rRNA gene was amplified from colony picks (Elliott et al., 2005) by PCR with global primers 27f and 1492r (Weisburg et al., 1991) to generate amplicons of approximately 1,499 bases (all primers were from Eurofins MWG Operon, Ebersberg, Germany). PCR was performed with 5

U Taq polymerase (BioTaq; Bioline, London, United Kingdom) per 100- $\mu$ l reaction mixture by using the buffer supplied. The PCR mixture also contained 2.5 mM MgCl<sub>2</sub> (Bioline, London, United Kingdom), 0.2 mM deoxynucleoside triphosphates (Bioline, London, United Kingdom), and each primer at 0.2  $\mu$ M. PCR mixtures were subjected to the following thermal cycling conditions: 94°C for 5 min; 29 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1.5 min; and 72°C for 5 min (Elliott et al., 2005).

The PCR products were cleaned and concentrated using SureClean PLUS kit (Bioline, United Kingdom). The full sequence (8-1492 nt; *E. coli* numbering) of the 16S rRNA gene of the strains was determined with big dye v3.1 on an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems, California, USA). Amplicons were sequenced from the 357F primer (David, 1991).

#### 6.2.5 Enzyme production at 4°C

Cold-active bacterial strain-specific extracellular enzymatic activities of amylase, lipase and  $\beta$ -galactosidase were assayed by transferring cultures grown in 96-well plates with the help of a microplate replicator onto 1:10 Tryptone Soy Agar at 4°C. 1:10 TSA supplemented with 1% (w/v) insoluble starch and detected as a clear zone around the colony (Tomova et al., 2013). After 4 weeks incubation, lugol was poured over the plate in order to increase the visibility of the halo. Lipolytic activity was tested by hydrolysis of 1% Tween 80 amended with 0.01%CaCl<sub>2</sub>.H<sub>2</sub>O and was seen as either a visible precipitate of the calcium chloride lipid complex around the colony, or as a clearing zone around a colony due to the complete degradation of the salt complex (Tomova et al., 2013). To screen for lactase ( $\beta$ -galactosidase) activity, 1:10 TSA was supplemented with 20  $\mu$ g/ml of X-gal, and was seen as development of blue colour in the colonies (Schmidt and Stougaard, 2010).

#### 6.2.6 Data analysis

All numerical data analysis was performed using R 3.3.2 (Team, 2013). Differences in CFU abundance between ice types were identified using Kruskal-Wallis analysis, and considered statistically significantly different when they showed p-values < 0.05.

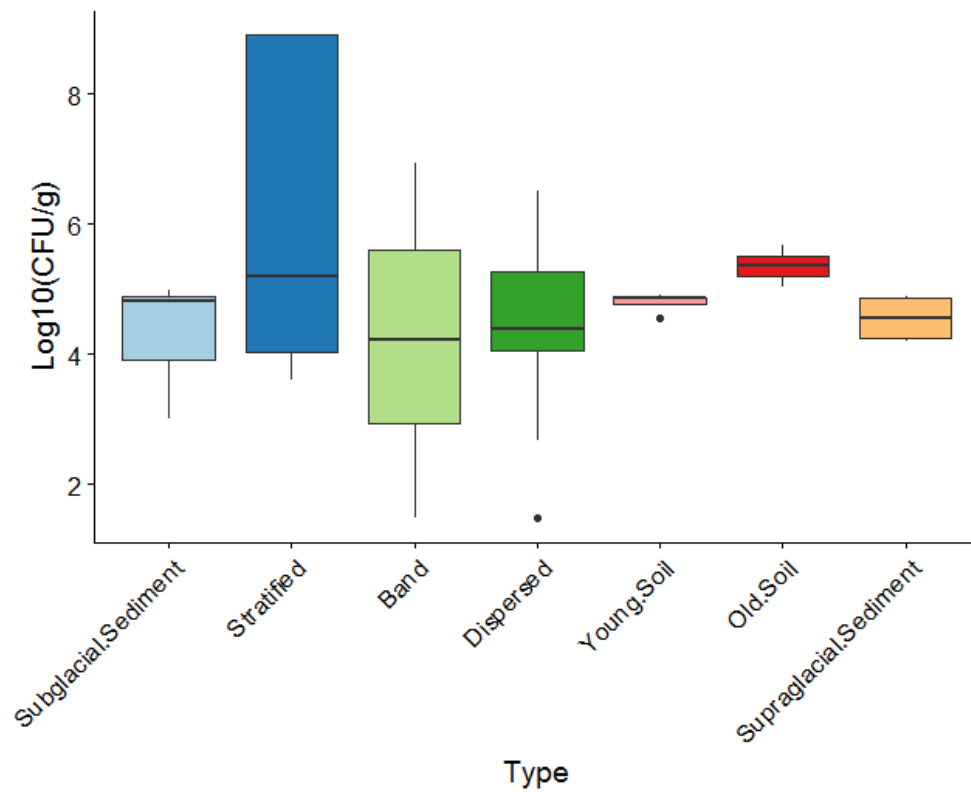
For all the microbial growth analysis, ggplots (Wickham, 2009) and gridExtra (Auguie, 2016) packages were used. Every experiment was run in triplicate in order to have statistically significant error bars.

DNA sequence analyses were performed using BlastN to determine their approximate phylogenetic affiliations (Altschul and Lipman, 1990). In addition, a phylogenetic tree was constructed using the neighbour-joining method (Jukes and Cantor, 1969; Kimura, 1980; Saitou and Nei, 1987; Jin and Nei, 1990) to enable comparison of the sequences, and visualisation of their relationship with selected sequences from GenBank. Sequences were aligned and gaps over 10 nts were manually removed. Stripping of the sequences was performed using the trimming tool in Mega7 Software (Kumar et al., 2016).

## 6.3 Results

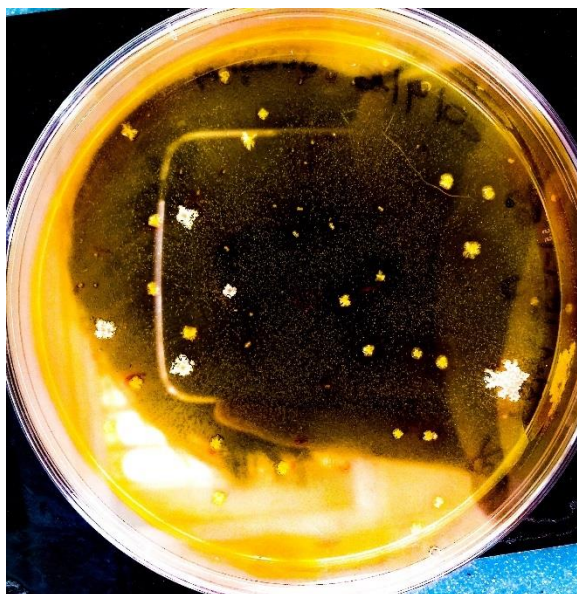
### 6.3.1 Microbial distribution in basal ice and the glacier foreland

Based on classical dilution plating and enumeration, basal ice exhibited the highest CFU g<sup>-1</sup> but also the highest variability: stratified ( $3.2 \pm 4.4 \times 10^8$  CFU g<sup>-1</sup>), debris band ( $1.2 \pm 3 \times 10^6$  CFU g<sup>-1</sup>), and dispersed ( $4.3 \pm 1.0 \times 10^6$  CFU g<sup>-1</sup>) (Fig 6.2) CFUs were statistically significantly higher in stratified facies than in debris bands (p-value=0.03) and dispersed facies (p-value=0.03). The lowest CFUs were attributed to supraglacial and subglacial sediments at  $4.5 \pm 3.3 \times 10^5$  CFU g<sup>-1</sup> and  $5.3 \pm 4.8 \times 10^5$  CFU g<sup>-1</sup>, respectively. Soil CFU g<sup>-1</sup> were higher than subglacial sediment (MOT and ST) and increased with soil age from  $6.5 \pm 2.0 \times 10^5$  CFU g<sup>-1</sup> to  $2.9 \pm 2.5 \times 10^6$  CFU g<sup>-1</sup> (in LIA and STOR).



**Figure 6.2. Culturable bacterial colony forming units (CFUs) on 1:10 TSA plates after 5 weeks incubation** expressed as  $\text{Log}_{10}$  for different ice types (stratified, band, and dispersed), sediment (subglacial and supraglacial) and soil (young and old) at Svínafellsjökull. The boxplots show the smallest and largest values, 25% and 75% quartiles and the median. The dots represent the outliers.

Growth in Ferrous Sulphate media did not show any colonies at 0% sea salts, but yielded some growth in some of the plates ( $\sim 10^3$  CFU g sediment) when the concentration was either 15% or 30% after a year-long incubation (Figure 6.3 and Table 6.2).



**Figure 6.3. Bacterial growth of potential chemolithotrophic species (Fe-oxidisers) in Ferrous Sulphate Medium after a year of incubation.** Different colour and morphologies can be observed, indicating probably different species.

**Table 6.1. Culturable bacterial colony forming units per gram of sediment (CFU g<sup>-1</sup>) on ferrous sulphate medium after 1 year of incubation at 4°C at 0, 15, 30% of sea salt concentration.** Samples labelled as B for debris bands, D for dispersed, and S for stratified. All basal ice samples are from Svínafellsjökull, except B5-16 and B6-16 from Kvíárjökull, and D7-16, D8-16, and S4-16, from Skaftafellsjökull.

Sample	0%	15%	30%
B1-16	Nd	nd	nd
B2-16	Nd	640	nd
B3-16	Nd	330	180
B4-16	Nd	170	nd
B5-16	Nd	470	nd
B6-16	Nd	nd	nd
D1-16	Nd	600	nd
D2-16	Nd	230	nd
D3-16	Nd	300	nd
D4-16	Nd	nd	190
D5-16	Nd	nd	nd
D6-16	Nd	nd	nd
D7-16	Nd	nd	nd
D8-16	Nd	nd	nd
S1-16	Nd	300	nd
S2-16	Nd	nd	nd
S3-16	Nd	nd	nd
S4-16	Nd	530	190

\*nd indicates that growth was not detected in the plates after a year long incubation at 4°C

### 6.3.2 Temperature-dependent Microbial growth

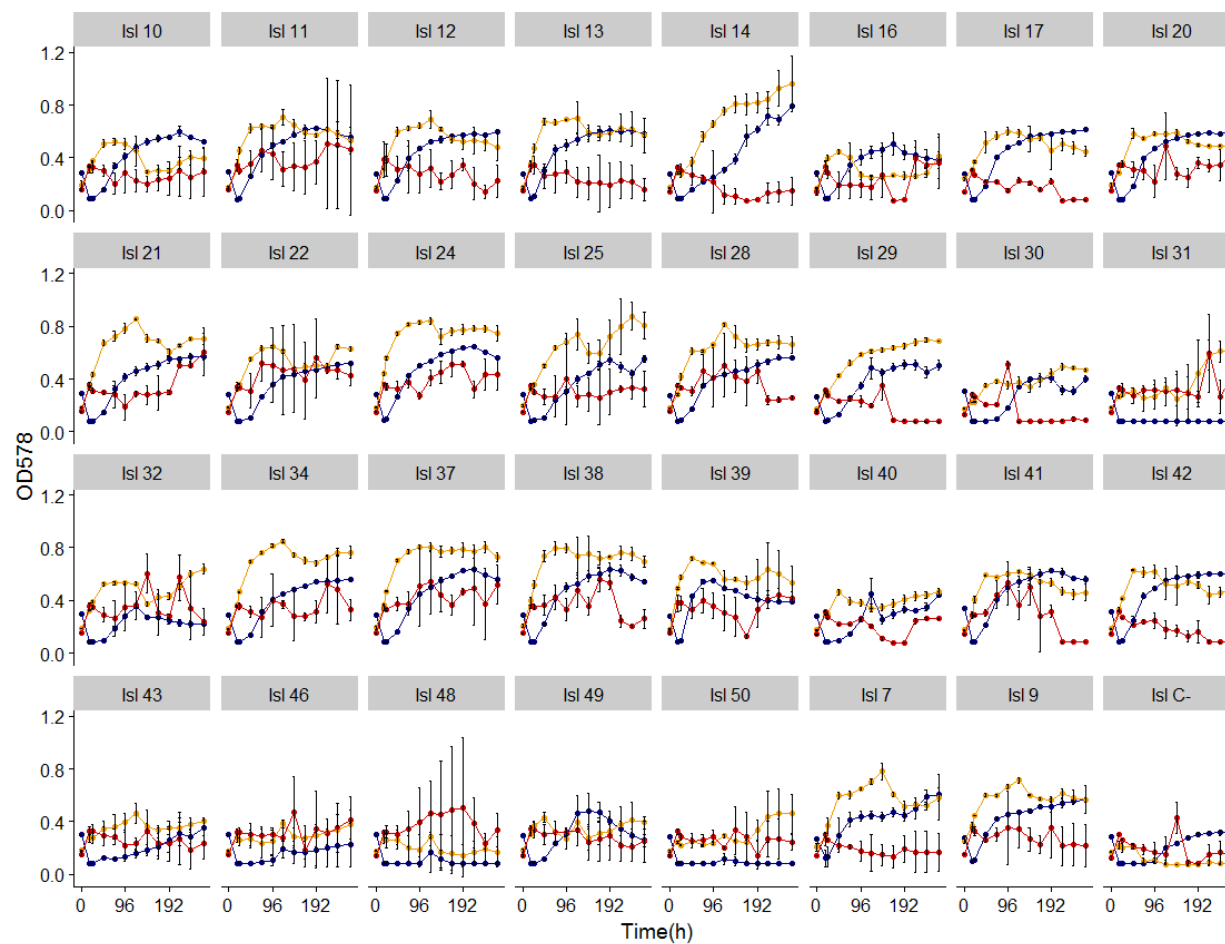
The majority of isolates from the 2012 campaign showed optimal growth at 25°C (71.0%) compared to 22.6% and 6.5% at 4°C and 37°C, respectively. Most isolates showed a psychrotolerant behaviour, being able to grow at 25°C and 4°C. The only isolate that showed a psychrophilic behaviour was Isolate 10, which exhibited inhibited grow at 25°C. (Fig 6.4).

### 6.3.3 Microbial growth at different salt concentrations

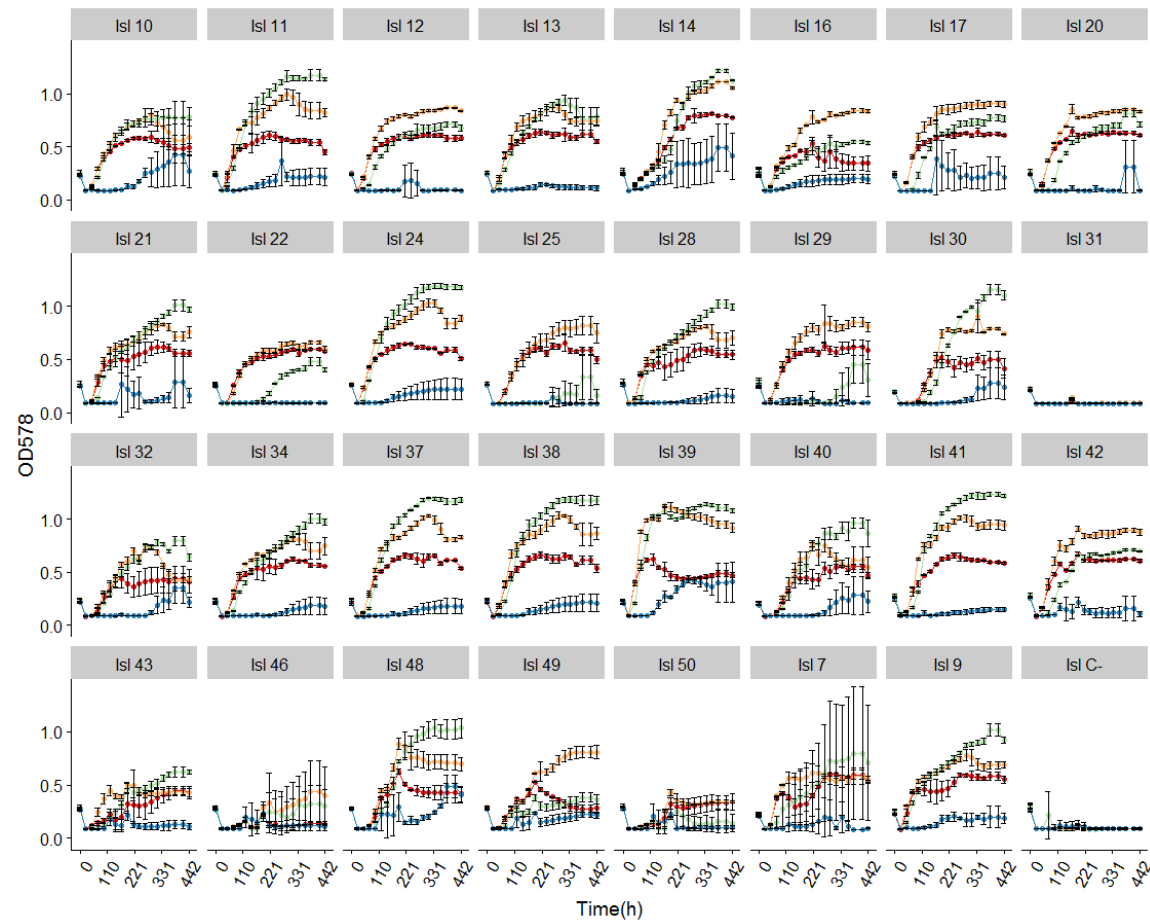
Growth of all test isolates was inhibited at 5% NaCl confirming a lack of true halophiles. The majority (63.3%) showed optimal growth at 2% NaCl compared to 33.3% at 0.5% NaCl. A single isolate (50) showed optimal growth in the absence of NaCl (Fig. 6.5).

### 6.3.4 Extracellular enzyme assay

Bacterial isolate-specific production and activity of three industrially relevant enzymes (lipase, amylase and  $\beta$ -galactosidase) on solid medium (1:10 TSA) at 4°C were characterised as follows: 54.4%, 35.1% and 31.6% of isolates showed lipase, amylase and  $\beta$ -galactosidase activities, respectively. 21.1% of the total number of isolates assayed did not show any activity for the enzymes evaluated in this study, 45.6%, 21.1% and 10.6% respectively showed single, double and triple activities. Isolates exhibiting all target enzyme activities were 4, 11, 26, 27, 38 and 51.



**Figure 6.4. Influence of temperature on microbial growth rates of different isolates from sediment and proglacial soil of Svínafellsjökull from the 2012 campaign, assessed as changes in the absorbance at 578 nm.** Isolates were grown in 96-well plates until up to 268 h and results are means calculated from triplicates. Standard deviation was plotted as error bars. Temperatures chosen for the assay were 4°C (blue), 25°C (yellow), and 37°C (red)



**Figure 6.5. Influence of salt concentration on microbial growth rates of different isolates from sediment and proglacial soil of Svínafellsjökull from the 2012 campaign, assessed as changes in the absorbance at 578 nm.** Isolates were grown in 96-well plates until up to 442 h and results are means calculated from triplicates. Standard deviation was plotted as error bars. Temperatures chosen for the assay were 4°C (blue), 25°C (yellow), and 37°C (red)

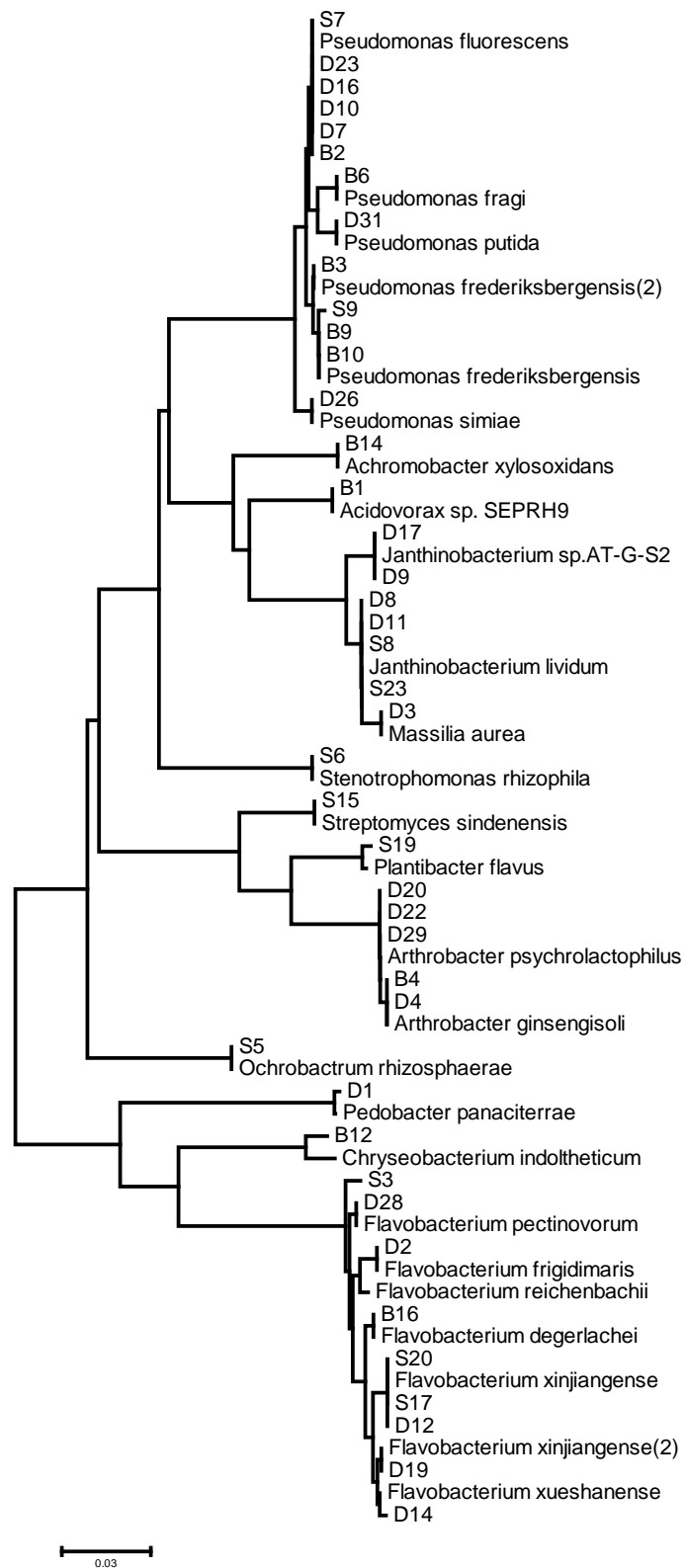


#### 6.3.5 Cultivable bacterial diversity in basal ice: dilution plating

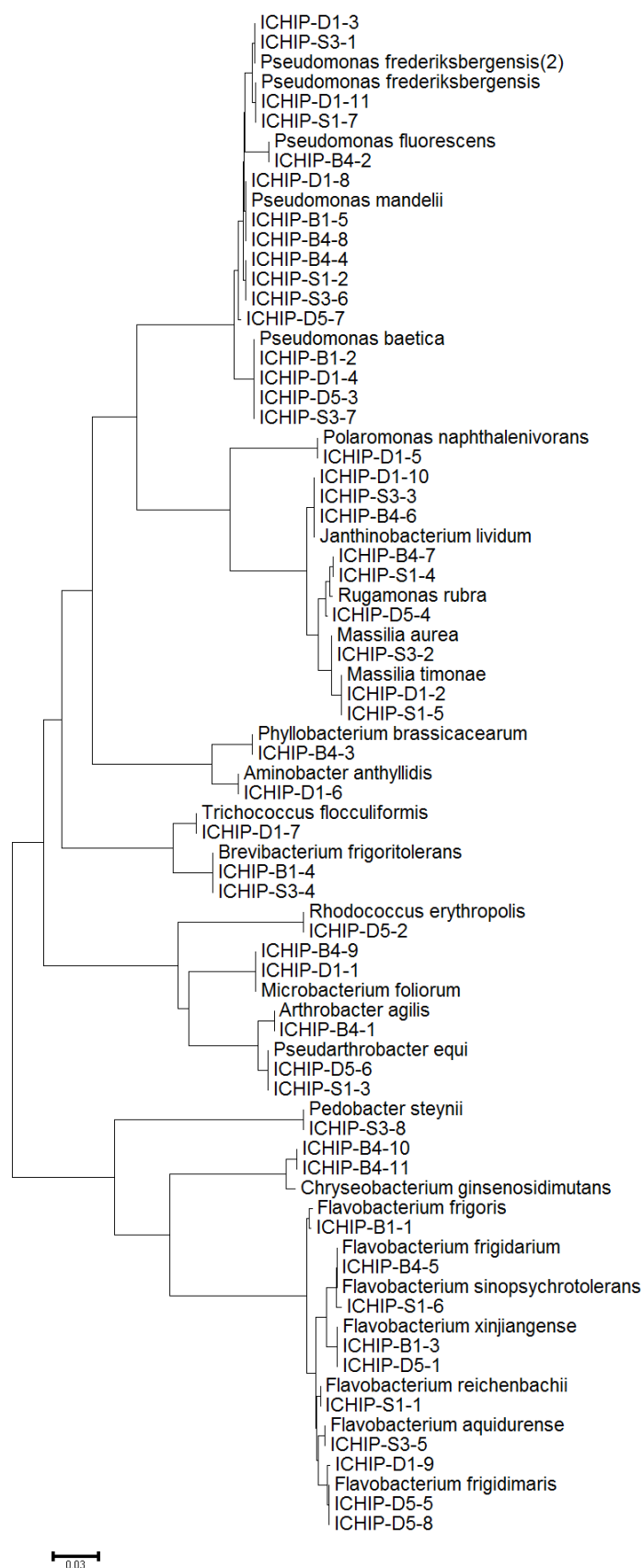
Based on morphological differences of isolates from the 2012 and 2015 campaigns following growth at 4°C, 44 isolates were subjected to phylogenetic 16S rRNA gene analysis (Fig. 6.6). Most of the isolates showed very high identity values with species previously isolated (99-100%), except S19 (98%), B12 (98%) and D15 (98%). No isolates were below the 97% threshold, which would have indicated a possible new species. From the total, 10 isolates sequenced from debris bands belonged to 6 genera, dominated by *Pseudomonas* (50%), and 10% were represented by *Achromobacter*, *Acidovorax*, *Arthrobacter*, *Chryseobacterium*, and *Flavobacterium*. 22 isolate sequences from dispersed facies again highlighted *Pseudomonas* abundance (27.3%), followed by *Flavobacterium* (22.7%), *Janthinobacterium* and *Arthrobacter* (18.2%), and *Massilia*, *Pedobacter*, and *Stenotrophomonas* (4.5%). 12 isolates were sequenced from stratified facies of which *Flavobacterium* was the most abundant genus (25.0%), followed by *Janthinobacterium* and *Pseudomonas*.

#### 6.3.6 Cultivable bacterial diversity in basal ice: ichip

Using ichip cultivation, 50 morphologically distinct isolates belonging to 16 genera from basal ice after incubation were identified from the 2015 campaign: 17 from debris bands, 19 from dispersed facies, and 14 from stratified (Fig 6.7). In all cases, *Pseudomonas* and *Flavobacterium* were the most abundant genera among the samples (*Pseudomonas*, debris band 29.4%, dispersed 31.6%, stratified 35.7%; *Flavobacterium* debris bands 17.6%, dispersed 21.1%, stratified 14.3%). Another three genera appeared in all samples: *Chryseobacterium* (debris band 11.8%, dispersed 5.3%, stratified 7.1%), *Janthinobacterium* (debris band 5.9%, dispersed 5.3%, stratified 7.1%), and *Massilia* (debris band 5.9%, dispersed 5.3%, stratified 7.1%). *Phyllobacterium* and *Arthrobacter* only appear in debris bands. *Polaromonas*, *Aminobacter*, *Trichococcus*, and *Rhodococcus* only appear in dispersed, and *Pedobacter* only appeared in stratified.



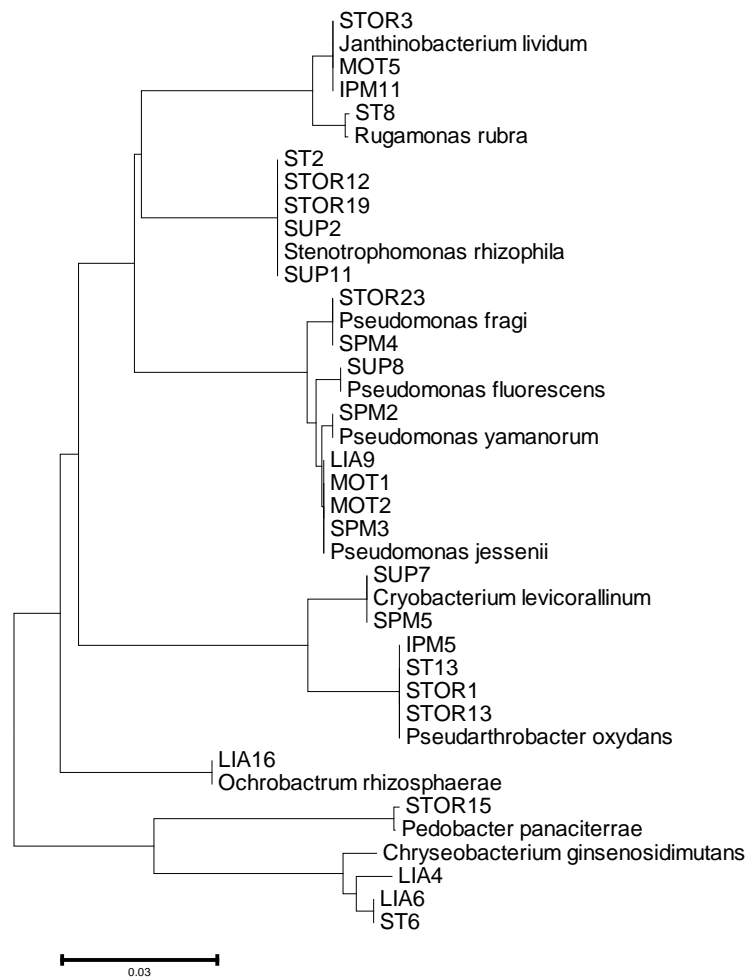
**Figure 6.6. Neighbour-joining tree of the 16S rDNA gene of the isolates obtained by traditional culturing from basal ice** using traditional spread plating of samples in 1:10 TSA after incubation for 5 weeks after sequencing using the 357F primer. The letter indicates the ice from which the sample was isolated: B, debris band; D, dispersed facies; and S, stratified facies.



**Figure 6.7. Neighbour-joining tree of the 16S rDNA gene of the isolates obtained from basal ice by culturing of ichip membranes after 10 days incubating in basal and plating of membranes in 1:10 TSA after incubation for 5 weeks after sequencing using the 357F primer. The letter indicates the ice from which the sample was isolated: B, debris band; D, dispersed facies; and S, stratified facies.**

### 6.3.7 Cultivable diversity of Svínafellsjökull's foreland

Samples from the 2012 campaign were cultured using traditional dilution plating of the supraglacial (SUP), subglacial sediment (ST and MOT), young soil (IPM and SPM) and old soil (LIA and STOR). 29 isolates were sequenced, belonging to 9 genera (Fig 8). 7 isolates from subglacial sediment were distributed at genus level as follows: *Pseudomonas* (n=2), followed by *Janthinobacterium*, *Rugamonas*, *Stenotrophomonas*, *Pseudarthrobacter*, and *Chryseobacterium*. Of the 4 isolates from supraglacial sediment: *Stenotrophomonas* (n=2), *Pseudomonas* (n=1), and *Cryobacterium* (n=1). The 6 isolates from young soil showed affiliation with *Pseudomonas* (n=3), *Janthinobacterium* (n=1), *Cryobacterium* (n=1), and *Pseudarthrobacter* (n=1). From the 12 old soil isolates, the following genera were identified: *Chryseobacterium* (n=3), *Stenotrophomonas* (n=2), *Pseudomonas* (n=2), *Pseudarthrobacter* (n=2), *Janthinobacterium* (n=1), *Ochrobactrum* (n=1), and *Pedobacter* (n=1). The only genus that appeared in common among all samples was *Pseudomonas*.



**Figure 6.8. Neighbour-joining tree of the 16S rDNA gene of the isolates obtained from supraglacial and subglacial sediment and proglacial soils by traditional culturing** using traditional spread plating of samples in 1:10 TSA after incubation for 18 months at 10°C after sequencing using the 357F primer. The cod indicates the sample origin: SUP (supraglacial sediment), MOT (melt-out till), ST (subglacial till), SPM (superior ridge of the seasonal push moraine), IPM (internal face of the seasonal push moraine), LIA (little ice moraine), STOR (Storalda Moraine)

## 6.4 Discussion

### 6.4.1 Bacterial cultivable diversity of basal ice, sediment, and foreland soil

The main aim of this microbiological study of Svínafellsjökull was the isolation and characterisation of the cultivable bacteria community inhabiting poorly microbiologically described glacier basal ice facies (debris bands, dispersed facies, and stratified facies), in conjunction with supraglacial and subglacial sediments, and forefront soils. The interface and interaction glacier-foreland have received growing attention in the last few years (Brown and Jumpponen, 2014; Lutz et al., 2014, 2015; Kim et al., 2017). Dilution plating culture of bacteria in routinely-used 1:10 TSA with antibiotic selection at 4°C for 6 weeks was performed in order to

identify bacteria microorganisms that are active in the basal ice environment using classical methodology. Although not exhaustive due to time and resource constraints, a total of 92 isolates belonging to 4 phyla: *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes* were cultured and phylogenetically characterised (16S) to genus/species level (Figs 6.6, 6.7 and 6.8). In this work, 29 isolates belonging to the *Pseudomonas* genus were isolated, belonging to 5 species, 4 of which are commonly found in soils: *P. fluorescens* (n=7), *P. putida* (n=1), *P. frederiksbergensis* (n=8), and *P. simiae* (n=1), *P. mandelii* (7), and *P. baetica* (Abdel Megeed and Mueller, 2009; Formusa et al., 2014; Pham et al., 2014; Bahena et al., 2015; Campos et al., 2015; Luján et al., 2015; Michelsen et al., 2015; Chong et al., 2016; Chatterjee et al., 2017; Suenaga et al., 2017). The genus *Pseudomonas* encompasses a large number of species (>200), which are known to be aerobic and some of them play very important roles in microbial-induced mineral weathering (Uroz et al., 2009). This genus is very cosmopolitan and is known to harbour species that are highly resistant to both internal and external stresses. In addition, *Pseudomonas* species produce a very wide range of bioactive compounds. Some species of *Pseudomonas* are very well recognised pathogens, for plants and animals (including humans) (Nikel et al., 2014). *Pseudomonas fluorescens* (n=7) is a plant growth-promoting rhizobacterium (PGPR), enhancing plant growth and triggering systemic resistance in plants (Bjorklof et al., 2003; Ye et al., 2014). The most similar strain found to that isolated in this work was previously isolated in hot springs, and some members affiliated with this species have shown thermotolerant capabilities (Kumar et al., 2014). In conditions of Fe scarcity, *P. fluorescens* and *P. putida* are able to produce siderophores, chelating agents that bind Fe, allowing bacterial uptake (Ye et al., 2014). *P. fragi* has been isolated from cold environments (Mei et al., 2016), such as Arctic soil, and is able to survive in cold temperatures. The 16S rDNA sequence with the highest homology to the *P. fragi* isolated in this work was found in industrially produced ice cubes (NCBI accession number: KX588591). Isolates affiliated with *P. fragi* are able to produce trehalose, which is a well-known protectant (Mei et al., 2016). *Pseudomonas frederiksbergensis* is a cold-tolerant bacteria able to oxidise sulphides (Adam et al., 2004) and long chain alkanes at low temperatures (Abdel Megeed and Mueller, 2009) having been isolated from cold environments such as soils of cold deserts (Yadav et al., 2015) and Arctic soil

(Lee et al., 2004); the most similar to the one found in this work was isolated from lake sediments. *Pseudomonas simiae* has also been isolated from cold Antarctic lake water (Martínez-Rosales and Castro-Sowinski, 2011). *Pseudomonas mandelii* is a psychrotolerant species, able to grow at 4°C to 30°C, but unable to grow at 37°C. It secretes lipolytic enzymes, which plays an important role in C-cycling (Jang et al., 2012). *P. mandelii* is able to survive in the absence of oxygen and possesses denitrifying capabilities (Verhille et al., 1999). *Pseudomonas baetica* is a fish pathogen able to grow between 4-30°C, and able to denitrify and produce H<sub>2</sub>S (López et al., 2012). Members of the genus *Pseudomonas* can actively weather minerals, such as phosphate or phosphate-containing minerals due to acid production, increasing its bioavailability, and iron-containing minerals, which can interact with the siderophores, removing it from the mineral matrix (Uroz et al., 2009). The presence of siderophore-producing *Pseudomonas* in basal ice could enhance the release of iron from minerals, such as haematite, entrapped in basal ice, which would be made readily available for other members of the microbial community. Upon release from basal ice to the glacier foreland, *Pseudomonas* can enhance weathering of P- and Fe-containing minerals, which would make conditions favourable for the colonisation of other pioneering communities, such as cyanobacterial species (Kaštovská et al., 2005, 2007), such as solidified lava fields (Kelly et al., 2014) or arid deserts (Lacap et al., 2011). In addition, *P. fluorescens* and *P. putida* are Plant Growth Promoting Rhizobacteria (PGPR) (Planchamp et al., 2014; Sivasakthi et al., 2014; Cole et al., 2015), and their identification strengthens the possibility that these PGPRs, upon release at the glacier margin, could facilitate plant colonisation immediately, or following further sediment weathering (Rime et al., 2016).

The most similar strain to the *Stenotrophomonas rhizophila* isolated from basal ice was found in the rhizosphere (GenBank accession no: KY474340.1), but this species appears to be ubiquitous in the environment (Wolf et al., 2002; Schmidt et al., 2012; Alavi et al., 2013). Members of this species have been recognised as PGPRs controlling the plant pathogenic fungal community (Schmidt et al., 2012), and have the ability to proliferate under salty conditions (3%), due to the production of

osmoprotectants (Schmidt et al., 2012). Isolates affiliated with this species have the ability to grow at 4°C (Wolf et al., 2002).

A total of 10 species belonging to the  $\beta$ -Proteobacteria group were identified. *Achromobacter xyloxdans* (n=1) is found normally in the plant phyllosphere, and can play a dual role - some strains of this species are plant pathogens (Spilker et al., 2012), whereas others have plant-beneficial PGPR potential (Jha and Kumar, 2009). The presence of nitrogenase activity allows members to fix atmospheric N<sub>2</sub>. N is a limiting factor for microbial and plant proliferation, and N fixation and transfer to the sediment has been identified as a very important step in the pedogenic process (Brankatschk et al., 2011). The strain of *Acidovorax wohlfahrtii* (n=1) isolated from debris band (Fig 6.6) that has highest homology to an isolate from Clausena (GenBank accession no: KC178583.1). Not much information about this species is available, but other members of this genus are known to be able to weather anaerobically using NO<sub>3</sub><sup>-</sup> as a terminal electron acceptor (Dippon et al., 2012; Pantke et al., 2012). Interestingly, *Rugamonas rubra* (n=3) (identity 98%), was originally isolated from cryoconite holes and glacier surfaces in Antarctica, but unfortunately there is not much information available for further ecological interpretation (Austin and Moss, 1986; Bowman and Deming, 2017). *Janthinobacterium lividum* (n=7), has been isolated from the upper surface of a glacier in India, but additionally, members of this species have been found in cold Alaskan soil (Schloss et al., 2010) and in Antarctica (Shivaji et al., 1991). Members of the *Janthinobacterium* genus isolate from glacier forefields are able to weather granite, releasing nutrients, especially Fe, whilst producing oxalic acid (Frey et al., 2010; Lapanje et al., 2012). Also, *Janthinobacterium* can produce HCN, which has a double effect. On one hand, it can interact with minerals, releasing nutrients, but also inhibits the cytochrome C of pathogenic fungi, acting as a PGPR. In addition, both oxalate and HCN can enhance mineral mobilisation (Frey et al., 2010). *J. lividum* produces a battery of secondary metabolites that have been reported to be toxic for other bacteria, viruses and protozoa (Schloss et al., 2010). *Massilia aurea* (n=2) has been isolated from a lagoon, but members of the genus *Massilia* have also been found in other environments such as sediments, soil and ice (Ofek et al., 2012; Shen et al., 2015). A different species of *Massilia* was



identified (n=2) in S1 and D1, and analysis of the 16S showed its affiliation with *M. timonae* isolated from soil, which can be an opportunistic pathogen in immunocompromised patients (La Scola et al., 1998; Faramarzi et al., 2009). An isolate affiliated with *Polaromonas naphthalenivorans* was found. This species encompasses facultative chemolithotrophs that were isolated initially from coal-tar waste. They are aerobic and their optimal temperature is 20°C, but are unable to grow at 30°C (Jeon et al., 2004). In addition, genes corresponding to the enzyme ribulose-1,5-biphosphate carboxylase/oxidase (RuBisCO) were identified in its genome, and fixation of CO<sub>2</sub> and H<sub>2</sub> oxidation have been reported in this species (Yagi et al., 2009).

Members of the large sub-phyla, Alphaproteobacteria were isolated. One isolate from a debris band (B4) affiliated with *Phyllobacterium brassicacearum* (Fig 6.7) has been described to exhibit PGPR capabilities, increasing plant stress resistance (Bresson et al., 2013). This species grows optimally at 28°C, but is unable to grow at 35°C (Mantelin et al., 2006). *Aminobacterium anthyllis* (n=1) is a nodulating rhizobacteria able to grow at temperatures of up to 37°C. It has been suggested to increase plant tolerance to metal contamination in the rhizosphere (Maynaud et al., 2012).

Two members of the phyla Firmicutes were isolated. A member of the species *Trichococcus flocculiformis* was identified in dispersed facies (D1) and is known as a facultative anaerobe that grows from 4-39°C (Scheff et al., 1984), with fermenting capabilities, generating lactate and acetate oxically, and lactate, acetate, formate and ethanol anoxically (Liu et al., 2002). The other *Firmicutes* found in B1 and S3 was identified as *Brevibacterium frigoritolerans* isolated from Saharan soils in Morocco (Delaporte and Sasson, 1967).

Importantly, 16 isolates were shown to have affiliations to the common soil filamentous phylum Actinobacteria. Six isolates from basal ice are related to three species isolated from soil: *Streptomyces sindenensis* (n=1), *Arthrobacter psychrolactophilus* (n=3), and *Arthrobacter ginsengisolu* (n=2). The latter has been isolated from cold soils (Siddiqi et al., 2014). A final actinobacterium isolated from stratified facies (Fig 6.6) was affiliated with *Plantibacter flavus* (n=1), which has been

isolated from lake sediment in the Himalayas (GenBank accession no: MF320300.1). *Streptomyces* appears in soil, water, and plant litter (Lingappa and Lockwood, 1962; Hsu and Lockwood, 1975; Goodfellow and Williams, 1983), but they have also been isolated from cold environments such as glaciers (Zhang et al., 2002) and cold soil (Malviya et al., 2009). In the soil, filamentous *Streptomyces* are very well recognised decomposers. In the presence of organic matter, rapid growth and colonisation is followed by profuse sporulation (Goodfellow and Williams, 1983). Members of the genus *Streptomyces* can degrade lignin and recalcitrant organic polymers (Dari et al., 1995). Members have been isolated from volcanic rock in Iceland and are involved in mineral release (Kelly et al., 2014). However, in this respect, there remains limited information on *Streptomyces sindenensis* identified in stratified facies (Fig 6.6). *Plantibacter* is a genus that typically appears associated with plants, associated with litter or with the rhizosphere, and the most similar strain to the one isolate in this work was isolated in a pond (Behrendt et al., 2002; Izumi et al., 2008). Three members of the genus *Arthrobacter* were isolated. Microorganisms belonging to this genus are able to survive at cold temperatures and are commonly found in cold soil communities (Bajerski, Ganzert, Mangelsdorf, Padur, et al., 2013; Dsouza et al., 2015). Some members of this genus have been reported to be able to weather minerals, such as hornblende (Kalinowski et al., 2000; Huang et al., 2014), releasing Fe. Similarly to some *Janthinobacterium*, members of the genus *Arthrobacter* are able to produce oxalic acid and HCN and attack granite, releasing nutrients (Frey et al., 2010). Both species identified in basal ice were originally isolated from soils and have been reported to grow at low temperatures: *A. psychrolactophilus*, 0-30°C and was isolated from soil (Loveland-Curtze et al., 1999) and *A. ginsengisoli* is able to grow between 5-30°C (Siddiqi et al., 2014), *A. agilis* grows optimally between 20-30°C (Koch et al., 1995) and has been described to be a PGRP by increasing plant root uptake of Fe (del Carmen Orozco-Mosqueda et al., 2013) and inhibiting fungal growth (Velázquez-Becerra et al., 2013). *Rhodococcus erythropolis*, like many members of this genus, has very powerful biodegrading capabilities, being able to degrade even very recalcitrant substances. Members of this genus have been isolated in the Arctic and Antarctica (Whyte et al., 2002; De Carvalho and Da Fonseca, 2005). The enzymatic battery of *R. erythropolis* allows attack of recalcitrant halogenated

compounds, and is able to remove sulphur from sulphur-containing compounds (De Carvalho and Da Fonseca, 2005). *Microbacterium foliorum* is a strict aerobe that is able to grow between 4 and 37°C, but not higher. It has been isolated from the phyllosphere of grasses (Behrendt et al., 2001), but also from cold proglacial soils, and individuals affiliated with this species were able to produce cold active hydrolases (Roohi et al., 2011). Not much information is available about the genus *Pseudoarthrobacter* but they have been found to be able to degrade contaminants such as lindane (Nagpal and Paknikar, 2006).

The only  $\alpha$ -Proteobacteria identified was closely related to *Ochrobactrum rhizosphaerae*, isolated from agricultural soil (Kämpfer et al., 2008). *Ochrobactrum* is a genus ubiquitously isolated from soil, but also the rhizosphere and plant tissues, water bodies, and even animals (Schloter et al., 2000; Bathe et al., 2006), and also in cold environments, such as snow in glaciers (Liu et al., 2009). Members of the genus *Ochrobactrum* have been reported to fix N<sub>2</sub> when they establish symbiosis with legumes (Ngom et al., 2004).

Fifteen isolates were affiliated with the *Bacteroidetes* phylum. One had 99% percent homology to *Pedobacter panaciterrae*, found in dispersed facies (Fig 6.6), isolated from soil (Yoon et al., 2007), and the other *Pedobacter*, isolated from stratified facies, was identified as *P. steynii* (Muurholm et al., 2007). The latter species was isolated from samples from a hard-water creek and showed growth between 10-30°C and is able to grow under microaerophilic conditions (Muurholm et al., 2007). One isolate was highly similar to *Chryseobacterium indoltheticum*, isolated from plants. *Pedobacter* comprises species that have been isolated from soil, commercial inoculums, and from cold environments such as cryoconite holes and glacial water. The one isolated in this work was found in soil from a ginseng field (Yoon et al., 2007). Members of the genus *Pedobacter* isolated from glacier forelands are able to weather minerals and release P and Mn (Frey et al., 2010). The genus *Chryseobacterium* comprises species that have been isolated from very different sources, including human samples, marine mud and sediment, fish (Jooste and Hugo, 1999), but also from cold environments such as Antarctica and Greenland (Bajerski, et al., 2013). *Chryseobacterium indoltheticum* grows between 20-30°C, but has not been described

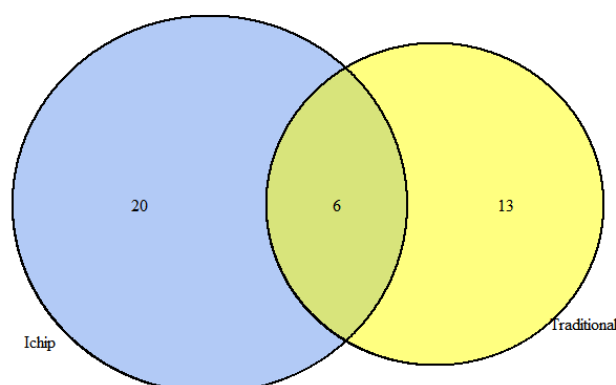
to grow at 4°C and it has been isolated from sea water (Campbell and Williams, 1951). A second species, *Chryseobacterium ginsenosidimutans* was isolated. Members of this species are able to grow between 10-37°C, but not at 4°C. Nineteen isolates were affiliated with the *Flavobacterium* genus. The genus *Flavobacterium* comprises several species that have been isolated from cold environments. *F. degerlachei* (n=1) was isolated from bacterial mats from Antarctica lakes samples, and it is able to grow between 5-30°C (Van Trappen et al., 2004), and *F. xinjiangense* (n=6), *F. xueshanense* (n=1, 99% identity), and *F. sinopsychrotolerans* (n=1) were isolated from Chinese glaciers (Zhu et al., 2003; Xu et al., 2011; Dong et al., 2012). These three last species are pure psychrophiles, and *F. xinjiangense* is unable to grow in temperatures over 20°C (Zhu et al., 2003), whereas *F. xueshanense* can grow in temperatures over 18°C. *F. sinopsychrotolerans* is able to grow up from 4°C to 25°C, and able to grow in anaerobic conditions (Xu et al., 2011). *F. frigidimaris* (n=3) was isolated from sea water in Antarctica, and it is able to grow between 2°C and 26°C (Nogi et al., 2005), whereas *F. frigidarium* (n=1) was isolated from marine sediment in Antarctica, and able to grow between 0-24°C (Humphry et al., 2001). *F. reichenbachii* (n=2, 99% identity) and *aquiderense* were isolated from hard water, and the former can grow at temperatures as low as 6°C (Ali et al., 2009) whereas *aquidurens* (n=1) can grow between 13 and 30°C (Cousin et al., 2007). Finally *F. pectinovorum* (n=1, 99% identity) was isolated from soils and it is able to grow between 0-30°C (Christensen, 1977).

#### 6.4.2 Comparison traditional methodology - ichip

The use of different methods led to the isolation of different species. Using traditional streak plating methodology, 19 different species were identified, whereas using isolation chips, 26 different species were isolated. Only 6 species were commonly isolated using both methods: *F. pectinovorum*, *F. reichenbachii*, *F. xinjiangense*, *J. lividum*, *M. aurea*, and *R. rubra*. (Fig. 6.9).

Nevertheless, ichip was only used in 6 samples and a comparison between both methods led to the identification of  $7.7 \pm 1.2$  species by ichip and  $3.2 \pm 2.2$  species by sample, based on 16S identification of colonies based on their morphology. This

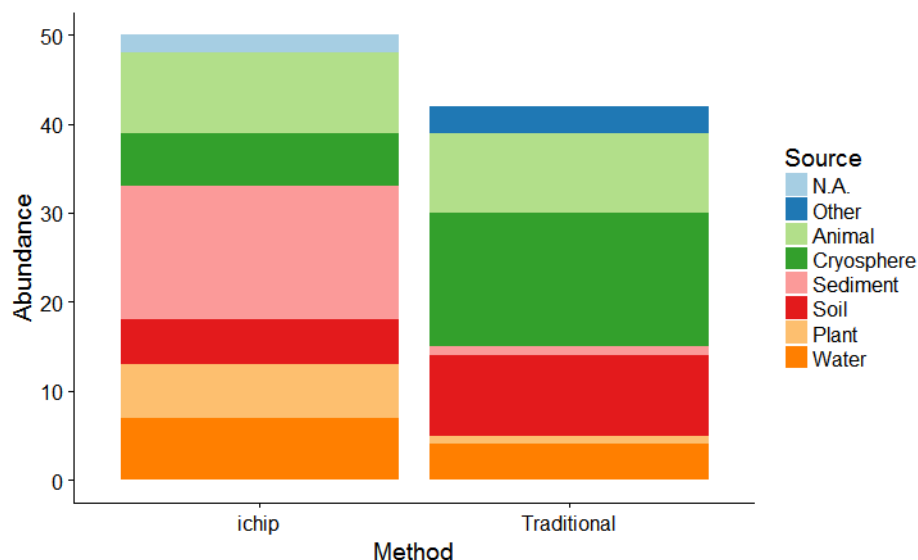
shows that ichips indeed lead to an increase in the recovery of species, as suggested by Nichols et al. (2010).



**Figure 6.9. Venn diagram showing the number of species isolated using different methodologies: ichip (n=26) in blue and traditional methodologies in yellow (n=19). The number of overlapping area represents the number of common species (n=6)**

Comparison of the isolates after a primary BlastN search revealed that using the traditional isolation methods, 30.8% of the isolates corresponded to isolates obtained from cryospheric environments, 23.1% isolated from soils, 15.4% from aquatic environments, 15.4% from animal samples, 3.8% associated with sediment samples, and 3.8% associated with plants. The remaining 7.7% corresponded to other sources, such as sludge. Using ichip cultivation, 18.5% of the isolates corresponded to samples from cryospheric environments, 18.5% from sediment, and 18.5% from water. Isolates from animals were represented in 14.8% of the isolates, and soil and plants corresponded to 11.1% each. 7.4% of the BlastN results did not show a match, and they have been marked as N.A. in Figure 6.10. The temperature conditions used for the isolation of microorganisms in this study (4°C) were optimised for the isolation of psychrophiles, which are known to grow in cold environments. The high abundance of soil microbiota in basal ice facies could be explained by the history of Svínafellsjökull. This glacier has been ~3.5 km shorter in the past (14<sup>th</sup> Century) (Ives 2007), and the foreland at that time was used for agricultural production or was forested with mountain birch. These vegetated and organic rich soils have since been

overridden by glacier advance between the 14<sup>th</sup>-19<sup>th</sup> centuries. It is therefore very likely that this soil and organic matter was entrained into the basal ice matrix.



**Figure 6.10. Isolation methodology-specific and environmental source relationships of identified bacteria** after 16S rDNA BlastN searches of all cultured isolates from 94 isolates at Svínafellsjökull. N.A. indicates that the deposited sequence returned in the analysis did not specify the isolation source.

#### 6.4.3 Potential role of cultivable microbiota

In oligotrophic environments, such as basal ice sediment (Skidmore et al., 2000), there is a need for chemolithotrophic species to proliferate and enrich the environment in C (Boyd et al., 2014). *Polaromonas naphthalenivorans* can oxidise H<sub>2</sub> and fix CO<sub>2</sub> acting as a facultative chemolithotroph (Jeon et al., 2004; Yagi et al., 2009) and was identified by 16S rDNA sequencing between the colonies grown in 1:10 TSA. In addition, a medium specific for Fe-oxidisers was made and some of the samples showed up to 6.4x10<sup>2</sup> CFU g<sup>-1</sup> sediment, which indicates that there can be a thriving community of Fe-oxidisers inhabiting basal ice. The enrichment in C in the sediment due to chemolithotrophic activity would allow the presence of heterotrophic communities (Boyd et al., 2014). In low C conditions, the ability to recirculate C and re-introduce it into the environment is highly important and species such as a *Streptomyces sp.* (Goodfellow and Williams, 1983), *Pseudomonas mandelii* (Jang et al., 2012), and *Rhodococcus erythropolis* (De Carvalho and Da Fonseca, 2005) can play this role, due to their high hydrolytic capabilities. Apart from C, heterotrophs need other nutrients present within substrate minerals (Hoffland et al., 2004; Uroz et

al., 2009), and these nutrients can be released as a result of microbial activity. In the literature it has been described how chelation by siderophores produced by *Pseudomonas fluorescens* or *putida* (Ye et al., 2014). or acid production, by species such as *Janthinobacterium lividum* (Frey et al., 2010) can release Fe from sediment. Other species such as *Thichococcus flocculiformis* can also produce acids by fermentation in anaerobic conditions, which not only can weather minerals, but also could serve as a source for methanogens (Scheff et al., 1984; Liu et al., 2002).

Microorganisms and sediment together with meltwater will be released from the ice matrix at the terminus, and deposited at the glacier margin (Rime et al., 2016; Toubes-Rodrigo et al., 2016). Under the current conditions of global warming, the process of glacial sediment, nutrient, and microbial release is likely to gather pace (Hood et al., 2015; Bradley et al., 2016; Hotaling et al., 2017). The deposition from glaciers of viable microbiota with weathering capabilities allows proglacial sediment to be efficiently incorporated into soil; proglacial soil development is not merely a function of external physico-chemical processes such as e.g. freeze-thaw, rain or wind erosion and weathering, as has traditionally been assumed (Bradley et al., 2016).

Toubes-Rodrigo et al., (2016) estimated that  $\sim 10^{16}$  cells  $\text{yr}^{-1}$  are being released from Svínafellsjökull to the glacier margin. Results from the study reported here targeting the same basal ice samples strongly supports the hypothesis that a significant proportion of these microorganisms are alive and viable. As discussed in this chapter, some of the microorganisms have weathering capabilities, and can release nutrients from the mineral matrix in the debris entrapped in basal ice.  $10^1$ - $10^6$  heterotrophic cultivable bacteria were isolated in this work, which represents a very small part of the total microbiota (Vartoukian et al., 2010; Pham and Kim, 2012). Some of the microorganisms identified in this work might be playing fundamental roles in the glacier margin upon release. It has been described that the first step for microbial establishment is the presence of a chemolithotrophic community, able to enrich the sediment in C, and as it has been suggested that this might be occurring in the basal ice, but also once that the sediment has been released from the glacier (Bradley et al., 2014). The second step is enrichment in nitrogen, and recently deglaciated soil have been found to be very poor in this element with a range of  $10^2$ - $10^3$  ppm (Bradley

et al., 2014; Vilmundardóttir et al., 2014). Some N fixers were identified among the samples, such as *Achromobacter xyloxidans*. The enrichment in C and N, linked to the release of nutrients from the minerals would make a suitable environment for the colonisation by plants (Kaštovská et al., 2005; Zumsteg et al., 2012; Brown and Jumpponen, 2014). The process of plant colonisation could be enhanced by PGPRs and some of the isolates found in this work such as *Pseudomonas fluorescens* (Ye et al., 2014), *Stenotrophomonas rhizophila* (Schmidt et al., 2012), *Phyllobacterium brassicacearum* (Bresson et al., 2013), or *Arthrobacter agilis* (del Carmen Orozco-Mosqueda et al., 2013).

#### 6.4.4 Succession in the cultivable microbiota in the foreland

In order to analyse the influence of the glacial microbiota in the glacier foreland, samples from supraglacial ice (SUP), subglacial sediment (ST and MOT), young soil (SPM and IPM), and old soil (LIA and STOR) were isolated (Fig 6.8). The two isolates analysed from supraglacial sediment were highly similar *Stenotrophomonas rhizophila* and *Cryobacterium levicorallinum*. *S. rhizophila* was also found in samples from sampled basal ice. One of the isolates corresponded to *Pseudomonas fluorescens* isolated from glacier ice and the other was *Cryobacterium levicorallinum*. This genus contains a high number of psychrophilic members isolated from glaciers, glacial and Arctic soils (Suzuki et al., 1997; Reddy et al., 2010; Liu et al., 2012). Isolates from subglacial sediment corresponded to *J. lividum* (n=2), *R. rubra* (n=1), *S. rhizophila* (n=1), *P. jessenii* (n=2), *Pseudoarthrobacter oxydans* (n=1), and *Chryseobacterium ginsenosidimutans* (n=1). *J. lividum*, *R. rubra*, *S. rhizophila* were also found amongst the basal ice samples. Additionally, other members of the genus *Chryseobacter* and *Pseudomonas* were identified in basal ice, although they do not correspond to the same species found in subglacial sediment. *C. ginsenosidimutans* has been reported from soils, and *P. jessenii* was isolated from industrially produced ice cubes (GenBank accession no. KX588595.1). *Pseudoarthrobacter* is a genus highly similar to *Arthrobacter* but not much information about this genus is available, making the ecological interpretation difficult.

Young soil isolates were identified as *J. lividum* (n=1), *P. fragi* (n=1), *P. yamanorum* (n=1), *P. jessenii* (n=1), *C. levicorallinum* (n=1), *P. oxydans* (n=1). The isolate found



here showed 100% homology to *P. yamanorum* isolated from deep sea sediment, but members of this species are psychrotolerant and have also been found in cold soils (Arnau et al., 2015).

Isolates from old soils corresponded to *J. lividum* (n=1), *S. rhizophila* (n=2), *P. fragi* (n=1), *P. jessenii* (n=1), *P. oxydans* (n=2), *Ochrobactrum rhizosphareae* (n=1), *Pedobacter panaciterrae* (n=1), and *C. ginsenosidimutans*. As mentioned before *S. rhizophila*, *P. panaciterrae*, *C. ginsenosidimutans*, are soil microorganisms, which are expected regarding the nature of the sample. The presence *J. lividum* would mean that the weathering still persists in old soil, which would help the vegetation to become established, releasing minerals, in addition to the PGPR species already mentioned.

## 6.5 Conclusions

Different cultivation methodologies were used in this work. Ichips led to the cultivation of a higher diversity of species than using traditional culturing by streak-plating. Nevertheless, only 6 species were found in common between the two methodologies, which means that traditional cultivation methodologies are needed in order to get a broad image of the community.

Stratified facies contained a significantly higher number of cultivable bacteria than the rest of the ice types, and higher than recently exposed soil. The release of sediment from basal ice would mean a significant change in the conditions, which would lead to a reduction of the diversity, associated with the lack of adaptation of the microbial community entrapped in basal ice. Nevertheless, due to progressive adaptation and external input, the diversity and microbial abundance will increase as the soil age increases.

Two genera dominated among the samples: *Flavobacterium*, which have been reported several times in glacier-derived samples, and that shows adaptations to these conditions; and *Pseudomonas*, one of the most cosmopolitan genera, with a very versatile metabolism, which allows them to thrive in adverse conditions.

Svínafellsjökull, as all Icelandic glaciers, has a history of advance and recession, and it has overridden soil in the last centuries. Both traditional and culturing methodologies showed a high abundance of soil-related species, supporting this observation. The role that the soil-related species found in this work is of high relevance for soil development: weathering and nutrient mobilisation, PGPR, nitrogen fixation. These microorganisms have been recovered from basal ice, showing that they are viable, and able to thrive in conditions likely to be found once released from the basal ice at the glacier margin. In addition, the majority of individuals corresponded to species found in cryospheric samples. So, unsurprisingly, most samples were psychrotolerant, which allows them to increase their colonising potential. Only 2 microorganisms had their optimal temperature at 37°C (Isolate 43 and Isolate 46), both isolated from old soil samples.

In summary, culture-dependant methods are biased, but still of high relevance, allowing to define the conditions for microbial survival and identification of isolated to species level, which increases the ecological interpretation of the data. Viable microbial communities are dominated by cryospheric-derived species, and soil species. The release of viable microbiota to the glacier margin will enhance the process of soil formation, and the community will change from a typical cryospheric microbiota to typical soil microbiota.

## 7. Discussion

Glaciers hold a position of great relevance amongst the biomes of Earth. Glaciers, occupying 11% (Hood et al., 2015) of Earth's total surface, together with permafrost play a significant role in the Earth system. Glaciers represent a major carbon store, estimated to be 6 Pg (Hood et al., 2015), more recently have been shown to harbour active ecosystems that complement physical activities significantly driving local biogeochemical cycles (Hodson et al., 2008; Anesio and Laybourn-Parry, 2012; Boetius et al., 2015).

The main focus of this PhD has been the characterisation of the geomicrobiology of the basal ice of Svínafellsjökull glacier, in order to understand the role of the microbiota in the basal ice layer, and the implication of it in weathering, and to an extent, in soil formation

The inter-disciplinary studies reported here (Chapters 3, 4 and 5, and 6) shed new light on, and extend current rudimentary understanding of basal ice. There is a discussion in every chapter about the results obtained, however this last final discussion aims to interconnect all the new information acquired in this PhD, aiming to show the relationship between the glaciology, chemistry, and geology from Chapter 3, with the microbial diversity from Chapter 4, the cell export calculated in Chapter 5, and the viable and cultivable microbiota identified in Chapter 6.

### 7.1 Basal ice as a microbial ecosystem

One of the objectives of this thesis targeted a physico-chemical evaluation of glacier basal ice to identify niche properties likely to support the microbial ecosystem (Chapter 3, Objective C). After enumeration of microbial cells in the basal ice (Chapter 4, Objective A), and enumeration of viable microbial cells (Chapter 6, Objective A), basal ice seems to be a viable environment for microbial survival.

In the limited number of studies investigating basal ice *per se*, microbes were visualised occupying localised "hotspots" characterised by the presence of liquid water between ice crystals (Mountfort et al., 1997) and around mineral sediment grains (Tung et al., 2006). Previous research by Mader et al., 2006, calculated the effective concentration of solutes in these brine veins, based on the concentration

obtained by bulk analysis and the diameter of ice veins. They found that the liquid water was highly enriched in solutes, due to solute exclusion during ice crystal formation. As such, the effective concentration of solutes in ice veins and mineral sediment films are, by definition, likely to be orders of magnitude higher than concentrations reported from the bulk analysis of the ice matrix (such as ICP-OES or IC) (Mader et al., 2006). The main focus of the chemical analyses of distinct basal ice facies was to identify and quantify elements and ions (e.g. Fe,  $\text{SO}_4^{2-}$ , Cl) that are crucial nutrients utilised by microorganisms to support metabolism and growth (Skidmore et al., 2000; Boyd et al., 2014) and thus to define a framework for microbial life.

#### 7.1.1 Ice matrix

Solutes in the ice matrix were very low, being the most abundant Na (~6-11 ppm), Ca (~5-7 ppm), Cl (~1-3 ppm), and S (~1-2 ppm) (Fig 3.6). Na and Cl are likely to be mainly derived from atmospheric deposition of ocean derived aerosols, which has been previously observed in Icelandic glaciers (Gíslason, 1990). The proximity of Svínafellsjökull to the sea (23 km) makes this process quite likely to happen. Ca has previously been described to be abundant in basal ice due to transfer from the bedrock (Hubbard et al., 2009). The high values of  $\text{Ca}^{++}$  have been previously described to be mobilised due to the presence of a water layer near the bedrock, favouring the dissolution of Ca-containing minerals (e.g. limestones) (Hallet et al., 1978). ICP-OES in the sediment revealed that Ca was the second most abundant of the analysed elements in sediment: debris band  $1.1 \pm 0.5$  ppm, dispersed  $2.0 \pm 1.6$  ppm, stratified  $1.3 \pm 0.3$  ppm (Fig 3.5). This would suggest that not only limestone can enrich basal ice in  $\text{Ca}^{++}$  but also volcanic rock (basalts and hyaloclastites majorly, Fig 1.12) would have a similar effect.

The relevance of S in microbial metabolism has been widely studied (Dahl and Friedrich, 2008), and besides being one of the fundamental elemental nutrients, it can be used as an energy source by chemolithotrophs when present in reduced forms such as sulphide, element sulphur or thiosulphate, or when oxidised to sulphate it can be used in anaerobic respiration (Dahl and Friedrich, 2008). The relative high abundance of sulphate can be associated to chemolithotrophs (Mitchell et al., 2013),

and its accumulation in the ice veins could be a consequences of the leaching from the mineral to the aqueous solution surrounding S-rich mineral grains (Vera et al., 2013).

Low concentration of inorganic N was detected (Fig 3.6), appearing as  $\text{NH}_4^+$  (0.02 ppm irrespectively of ice type) and  $\text{NO}_3^-$  (0.4-0.6 ppm).  $\text{NO}_2^-$  values were below limit of detection for most of the samples, except debris bands B1, B2, and SC DB (<0.01 ppm), and stratified facies S1, and S2 (0.01 ppm). Similarly to S, reduced N species, such as  $\text{NH}_4^+$ , or partially oxidised species such as  $\text{NO}_2^-$  can be used by groups of chemolithotrophs (ammonia oxidising bacteria – AOB, and nitrite oxidising bacteria – NOB) as energy substrates (Telling et al., 2012; Lutz et al., 2015).  $\text{NO}_3^-$  on the other hand, can be used by facultative and obligate anaerobes as an terminal electron acceptor in anaerobic respiration (Telling et al., 2012). Mn and Fe can be utilised when oxidised can be used as TEA in anaerobic respiration, and Fe(II) can be oxidised by chemolithotrophs. However, the values of these elements were very low in the ice matrix: Fe (0.04-0.07 ppm), and Mn (0.005-0.008 ppm)

#### 7.1.2 Sediment

Total carbon concentration in the sediment of the distinct basal ice facies (1046 ppm for debris bands, 1748 ppm for dispersed, and 2102 ppm for stratified) was found to be very low and comparable to e.g. hyperarid deserts (Valdivia-Silva et al., 2012). In order to maintain an active ecosystem, C-fixing microbes are crucial because of the absence of light in the subglacial environment, C-fixation cannot rely on photosynthesis but must be driven by chemolithotrophy instead (Amend and Teske, 2005)(Parnell et al., 2002; Tyler et al., 2002).

In their review, Hood et al., (2015), indicated that the highest abundance of C in glacial systems were found in cryoconite holes ( $\sim 1.5 \text{ mgC L}^{-1}$  for the Antarctica Ice Sheet,  $\sim 0.8 \text{ mgC L}^{-1}$  in mountain glaciers), followed by basal ice. Maximum recorded values in basal ice reached  $\sim 40 \text{ mg L}^{-1}$ . In order to obtain a comparable value total C-concentration expressed in ppm was converted to  $\text{mg g}^{-1}$  of sediment and multiplied by the concentration of sediment in basal ice in  $\text{g L}^{-1}$ . Values ranged from  $31.5 \pm 31.1 \text{ mg L}^{-1}$  for dispersed,  $434.9 \pm 588.9 \text{ mg L}^{-1}$  for debris bands, and  $818.4 \pm 353.4 \text{ mg L}^{-1}$  for stratified, highlighting significant basal ice facies specific C content

In order to estimate the microbial abundance in the basal ice, quantification of cell content directly (Table 4.1) and indirectly via estimates based on extracted DNA concentration were compared (Table 4.2). Based on direct counts microbial cell estimates for debris bands, dispersed and stratified basal ice facies were  $1.6 \pm 0.3 \times 10^7$ ,  $1.4 \pm 0.6 \times 10^7$  and  $1.3 \pm 0.3 \times 10^7$  cells  $g^{-1}$ , respectively. By comparison, DNA concentration for the respective basal ice facies were  $2.8 \pm 0.7$ ,  $2.1 \pm 1.3$  ng  $g^{-1}$ ,  $17 \pm 3.3$  ng  $g^{-1}$ . For biosphere DNA modelling, (Landenmark et al., 2015) had used the estimate of DNA-C representing 3% of total cell C. Furthermore, (Whitman et al., 1998) had estimated that in oligotrophic environments, such as basal ice (Margesin and Miteva, 2011; Montross et al., 2014), the C content in bacterial cells was 20 fg. From these data, estimation of cells based on DNA concentration yielded  $4.7 \pm 1.1 \times 10^6$ ,  $3.5 \pm 2.1 \times 10^6$  and  $2.8 \pm 0.6 \times 10^7$  cells  $g^{-1}$  for debris bands, dispersed and stratified facies, respectively. Regardless of the method and basal ice facies, all counts were in the range  $10^6 - 10^7$  cells  $g^{-1}$  of sediment. The significantly higher presence of microbial cells associated to sediment compared to the cell content in the matrix would mean that higher debris content would mean higher cell content in the basal ice as observed by (Montross et al., 2014).

In order to estimate what fraction of the C identified in sediment by LECO analysis, microbial C calculated based on DNA content using the 3% conversion factor (Landenmark et al., 2015) showed that only a small fraction of the total C is associated with microbial-C (0.07% for debris bands, 0.01% for dispersed, and 0.07% for stratified). This difference can be readily explained when consideration is given to Svínafellsjökull's terminus position. Svínafellsjökull, as all Icelandic glaciers, has experienced both periods of advance and recession (Hannesdóttir et al., 2015) and (Ives, 2007), based on historical records, found that Svínafellsjökull's terminus was approximately 3.5 kilometres higher than the current position in the 14<sup>th</sup> century. The extended foreland was used for agriculture, indicating that the C-content of the sediment was higher, in order to sustain agriculture. This C rich foreland soil could later have been entrained during the advance of the glacier and so contributing to elevated C content of the basal ice. Bulk analysis by (Lawson et al., 2015) found that basal ice sediment from glaciers from Antarctica, Svalbard, Canadian Arctic and the

Alps, also contained C in the debris particles, but the actual concentration of C was very low (>0.6%), and the labile carbon in these particles, measured as percentage of carbohydrates was very even lower (>0.5% of the total organic carbon).

Despite the overall low C content in basal ice, the carbon that is present might be playing a very important role in sustaining a basal ice microbial ecosystem, not reliant on the oxidation of C, but on chemosynthesis (Reysenbach et al., 2000; Chapelle et al., 2002; Tyler et al., 2002; Jones and Bennett, 2014). The in situ XRF data (Fig 3.3) generated highlighted that the most abundant element in basal ice was Si and subsequent SPA analysis confirmed that sediment derived from almost all basal ice samples was dominated by silicates (Fig 3.7), and although silicates *a priori* are not a mineral very prone to microbial colonisation (Mitchell et al., 2013), their presence, as the presence of other minerals, guarantee liquid water films surrounding the grains in which microorganisms can thrive (Tung et al., 2006). In addition, silicates often contain impurities or inclusions in their crystalline matrix that can serve as a key microbial nutrient source – for example, olivine, which contains Mg and Fe (Bennett et al., 2001; Rogers and Bennett, 2004). Silicates in aqueous conditions can lead to the production of H<sub>2</sub> and this process has been observed in aqueous subglacial environments (Telling et al., 2015). The H<sub>2</sub> produced by this process can then be oxidised by chemolithotrophic species (H-oxidisers) or hydrogenotrophic methanogens (Price, 2009).

Fe was the second most abundant element and was the most abundant metal identified in the XRF and ICP-OES analyses, respectively (Figs 3.3 and 3.5). Fe-rich mineral were very abundant among the minerals analysed by SPA (~5%) (Fig 3.7) but Fe representation in the ice matrix was negligible (Fig 3.6). These results suggest that Insoluble Fe (III) in the fully oxidised state, acts as a terminal electron acceptor in anaerobic microbial respiration (Nixon et al., 2017). In addition, microorganisms can solubilise Fe by reducing the pH surrounding the grains, by H<sup>+</sup>, production of acids (citric and oxalic) (Hoffland et al., 2004), or production of siderophores (Uroz et al., 2009). When reduced to Fe(II) as a result of the microbial respiration, or by the exposure of fresh mineral associated to glacial flow (Telling et al., 2015), Fe can be utilised by Fe-oxidisers. Evidence has been presented where bacteria and archaea

attached to Fe-containing minerals (Tung et al. 2006; Mitchell et al. 2013) showed a strong correlation between mineral Fe content and microbial content.

S values in sediment were found to be low, yet above detection limits in the ICP-OES analysis (0.09-0.19 ppm) (Fig 3.5) analyses and S was detected via XRF (Fig 3.3), which contrasts with the relative high amount of sulphate in the ice matrix (Fig 3.6). This differential would suggest that reduced species of S are rapidly oxidised and the resultant  $\text{SO}_4^{2-}$  leaches to the ice matrix. Oxidation of S have been identified previously in subglacial environments as a driver for chemolithotrophy (Skidmore et al., 2000; Mikucki and Priscu, 2007).

Al values by XRF were the fourth most abundant element (0.2-0.9%) in basal ice (Fig 3.3), and the third most abundant element in the sediment fraction analysed by ICP-OES (1.0-1.6 ppm) (Fig 3.5), but an order of magnitude lower in the ice matrix (0.15-0.2 ppm) (Fig 3.6). The co-occurrence of Al with Si ( $R^2=0.99$ ) in the XRF (Table 3.2) would suggest that Al appears as part of silicates, dominant minerals in the SPA (Fig 3.7). Al has been described to have toxic effects on cells, by interfering with the Fe homeostasis, and disrupting fundamental metabolic activities, such as the tricarboxylic acid cycle and oxidative phosphorylation (Lemire et al., 2010). Nevertheless both bacteria (Lemire et al., 2010, Illmer and Erlebach, 2005) and fungi (Tani et al., 2008) have developed mechanisms to reduce the toxicity of this element. Al can be released from the mineral matrix due to acidification of the environment, especially associated to sulphate production (Bigham and Nordstrom, 2000; Hao et al., 2010), which can be induced by microbial activity, and microbial activity has been suggested to generate bauxites (Hao et al., 2010). In addition, as mentioned previously, significant amounts of sulphate were found in the ice matrix (Fig 3.6), which would suggest that Al mobilisation could occur within the basal ice layer. Same as Al, Ti is also a toxic element (Silver and Phung, 2005) and appeared in significant amount in the XRF analysis (0.001-0.24%) (Fig 3.3). This result is not surprising since volcanic deposits present significant amounts of Fe-Ti oxides (Carmichael, 1966; Carmichael and Nicholls, 1967), and correlation analysis showed that Fe and Ti co-occurred in the samples analysed by XRF ( $R^2 = 0.983$ ) (Table 3.2), being the second strongest correlation. Although described as toxic (Silver and Phung, 2005), some



orders such as *Nitrospirales* have been described to thrive in the presence of Ti, and this element could be one of the shaping agents of the microbial community (McCluskey, 2016).

Cl appeared amongst the most abundant elements in the XRF analysis (0.3-0.5% in basal ice) (Fig 3.3) and in the IC analysis of the ice matrix (1.2-2.5ppm) (Fig 3.6), but presence of halides was not detected by SPA (Fig 3.7) suggested that it appeared dissolved as part of the liquid vein systems and surrounding grains (Mader et al., 2006). Cl can be used in anaerobic respiration (dehalorespiration) by certain autotrophic genera, such as *Dehalococcoides* (Lorenz et al., 2000). This genus can transfer electrons to chlorobenzenes, whilst using H<sub>2</sub>, which can be produced by chemical reactions of silicates in aqueous environments (Telling et al., 2015), as an electron donor in their metabolism (Lorenz et al., 2000).

Divalent cations, especially Ca<sup>++</sup> and Mg<sup>++</sup>, are associated to weathering from the subglacial sediment and incorporated into the basal ice layer (Hubbard et al., 2009). Both elements were amongst the most abundant in the samples analysed by XRF (Ca: 0.8-1.2% and Mg: 0.1-2.0%) (Fig 3.5). Results showed that Ca<sup>++</sup> was more abundant in the ice matrix than in the sediment (Fig 3.6), nevertheless Ca-rich minerals were detected by SPA (Fig 3.7). Enrichment of Ca in basal ice has been previously observed, linked to an enrichment in Mg (Hallet et al., 1978), and in the presence of sulphate, these two elements have been described to precipitate forming biogenic minerals (Bontognali et al., 2014). Although Ca and Mg are not directly used in redox process in order to obtain energy (chemolithotrophy) or as a terminal electron acceptor (respirations) microorganisms require of these two elements for their normal functioning, acting as secondary messengers (Ca<sup>++</sup>) (Domínguez et al., 2015) as well as cofactor in enzymes of vital importance (Yong et al., 2014, Knoop et al., 2005). Similarly to Ca, K can be accumulated inside of bacteria cells, and can act as an intracellular cytoplasmic-signalling molecule (Epstein, 2003), and therefore, playing fundamental role in the microbial metabolism. K appears as part of silicates, as an impurity intercalated in the SiO<sub>2</sub> matrix (Uroz et al., 2009), and correlation analysis showed that K and Si co-occurred in the XRF analysis ( $R^2 = 0.952$ ) (Table 3.2), suggesting that similarly, K in basal ice forms part of silicates. Microorganisms can

release this fundamental cation from the mineral matrix, making K<sup>+</sup> available for their metabolism (Hoffland et al., 2004; Uroz et al., 2009).

P value were very low in basal ice as shown by XRF analysis (0.001-0.042%) (Fig 3.3), ICP-OES of the sediment (0.05-0.11 ppm) (Fig 3.5) and ice matrix (0.02-0.03 ppm) (Fig 3.6). P is a major growth-limiting nutrient and tends to appear in an insoluble phase, making its bioavailability even lower (Mohammadi, 2012). Fungi have been proved to be major players in the solubilisation of P (e.g. *Aspergillus*), and also some bacterial genera (e.g. *Pseudomonas*) can solubilise this element (Hoffland et al., 2004; Stibal, Tranter, Telling, et al., 2008; Mohammadi, 2012). Among the processes implicated in the solubilisation of P, acidification is highly important, which helps to the mobilisation of P as phosphate (Mohammadi, 2012). No phosphates were identified in the SPA analysis (Fig 3.7), however, previous research in cryospheric environments (cryoconite holes) suggested that P, as phosphate, can bind with minerals containing Fe and Al (Stibal, Tranter, Telling, et al., 2008), and both of elements were abundant in the performed analyses.

The high numbers of microbial cells, the presence of a chemistry and mineralogy compatible with life and the recovery of viable cells from basal ice suggests that basal ice in Svínafellsjökull represents a conducive to supporting primary microbial producers. The high abundance of sequences affiliated with Fe-oxidising bacteria, the relative high abundance of Fe-containing particles, and the presence of viable and cultivable Fe-oxidising bacteria, seems to indicate that Fe oxidation is the main driver for C-input in the subglacial environment, sustaining the microbial ecosystem in basal ice.

## 7.2 Relationship between microorganisms and mineral content in basal ice

The high microbial total and viable cell counts (Chapter 4, Objective 1, and Chapter 6, Objective 1), distinct mineralogy and elemental status of basal ice at Svínafellsjökull strongly supports an environment where life is likely to thrive, despite the low C content and the absence of light. The aim of this section, based on the analysed mineralogy and chemistry of basal ice (Chapter 3, Objective 1) and tied to data on microbial identity (Chapter 4, Objective B) and abundance, is to establish a basic

model of the interactions between microbiota and the mineralogy and chemistry of the basal ice layer.

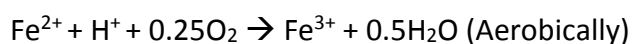
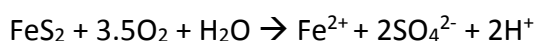
Microbial count data revealed that microorganisms attached to entrained sediment minerals were more abundant than in the ice matrix by 2 orders of magnitude (Fig 4.1). This emphasises the central importance of mineral-microbe interactions in supporting a productive basal ice ecosystem under conditions of autotrophy and C oligotrophy. The most abundant minerals, as previously mentioned, were silicates (Fig 3.7). Previous literature has showed that silicates can be weathered by certain microorganisms to release nutrients (Rogers and Bennett, 2004; Popa et al., 2012). Bacteria closely affiliated with some of the individuals isolated from Svínafellsjökull that are known to weather silicates include *Pseudomonas*, *Janthinobacterium* and *Pedobacter* (Fig 6.6, 6.7, and 6.8). The fact that, in this study, isolates of these genera were isolated under low nutrient conditions and temperatures (4°C) suggests silicate weathering capacities extend to psychrophilic communities able to survive in basal ice. These psychrophiles could be playing a very important role weathering silicates, releasing the nutrients entrapped in the crystalline matrix, by organic acid and siderophore production, making them readily available for supporting a more diverse microbial community (Uroz et al., 2009). Moreover, under aqueous conditions, silicates can release H<sub>2</sub> (Telling et al., 2015) that can be used by hydrogen-oxidisers (such as *Acidovorax*) (Lee et al., 2015) and methanogens (such as members of the phylum *Euryarcheota*) (Boyd et al., 2010; Stibal, Hasan, et al., 2012)

Fe is the fourth most abundant element of the lithosphere and it has been suggested to play a critical role in the redox chemistry in subglacial environments (Mikucki et al., 2016). The chemical analyses of basal ice in this study suggests that Fe predominantly exists as precipitated Fe(III). However, under anoxic conditions that are likely to persist in subglacial environments (Yde et al., 2010; Stibal, Hasan, et al., 2012), anaerobic microbial respiration will be selected for targeting the oxidation of Fe(III) to Fe(II). Under these conditions, Fe reducers are able use Fe(III) of minerals such as goethite, hematite, or magnetite (Nixon et al., 2017), identified in the subglacial environment by Raman spectroscopy, for growth metabolism and generating Fe(II). Fe reducing bacteria have been observed in subglacial

environments before (Foght et al., 2004), and members of genera able to reduce Fe(III) have been identified in this basal ice study, e.g. *Acidiferrobacter*, *Geothrix*, or *Geobacter* as well as some sulphate reducing bacteria (Weber et al., 2006) (Fig 4.3). On the other hand, the presence of reduced Fe(II) produced by respiration of Fe(III) containing minerals, or freshly exposed minerals prove that Fe-oxidisers, such as *Thiobacillus*, *Gallionella*, *Acidiferrobacter* can thrive, as evidenced in significant abundance of 16S sequences affiliated with Fe oxidisers in bacterial community inhabiting basal ice (5.5% on average, but reaching over 14% in some samples) (Fig 4.3). *Thiobacillus* was abundant among the basal ice samples, which is not surprising, considering the conditions identified in basal ice would select for proliferation of members of this genus known to be facultative anaerobes and acidophiles. In contrast, identified *Gallionella*, are strictly aerobic Fe oxidisers. Co-occurrence analysed by network analysis (Fig 4.7) suggests that members appear with *Acidiferrobacter* which is also a Fe-oxidiser, but can operate under anaerobic conditions reducing Fe(III) (Hallberg et al., 2011). A symbiotic relationship between members of both genera growing on the surface of Fe-particles could be seen as very mutually beneficial. Hypothetically, the Fe-rich particle would be enveloped by a Fe-oxidising biofilm with the exterior occupied by the oxygen requiring aerobe, *Gallionella*. As a result of oxygen consumption and respired CO<sub>2</sub>, oxygen will be depleted rapidly, and thus does not diffuse to the inner zone of the biofilm, where *Acidiferrobacter* could proliferate, oxidising Fe in order to obtain energy, but reducing it simultaneously to respire. Fe(II) could leach towards the outer part of the biofilm where *Gallionella* is present, using its oxidation as the energy source. On the outer surface of the biofilm, other aerobes, such as *Maritimonas* can proliferate, using the C- fixed by the chemolithotrophic members of the biofilm.

Although a low abundance of sulphur-containing minerals was detected among the samples analysed by SPA (<1%) (Fig 3.7), high sulphate levels were characterised in the IC analysis (Fig 3.6). Sequences affiliated with S-oxidisers, e.g. *Thiobacillus*, *Thiotricaceae*, were significantly abundant among the samples (average 12.2%, but in some of the samples the value reached over 25% of the total microbial community) (Fig 4.3). In cases where S-oxidisers are active in basal ice, with regards of the high

abundance for S-oxidisers detected, available S reduced species, such as sulphides, elemental sulphur, or thiosulphate, would rapidly be oxidised to sulphates, following the reactions as described in Sánchez-Andrea et al. (2011) as follows:



Progression of these reactions leads to an acidification of the environment driving the production and release of  $\text{H}^+$ . Although pyrite has been reported in the nearby Skaftafell area (Fairchild et al., 1999), no pyrite was detected amongst the Svínafellsjökull basal ice samples in this study. Nevertheless, some of the genera identified have been found to be S-oxidisers, such as *Thiobacillus*, *Gallionella*, *Acidiferrobacter*, the family *Thiotricaceae* (Taylor and Hoare, 1971; Lütters-Czekalla, 1990; Hallbeck and Pedersen, 1991; Kelly et al., 2005; Hallberg et al., 2011) (Fig 4.3). The presence of members of these genera does strongly support pyrite availability in the basal ice environment. There is previous evidence of biological sulphate production in subglacial environments (Sharp et al., 1999; Bottrell and Tranter, 2002; Mikucki and Priscu, 2007). The microbial oxidation of sulphides generates secondary minerals such as jarosite, goethite, schwertmannite, ferrihydrite (Lu and Wang, 2012). Raman analysis performed in this study confirmed some of these minerals, supporting the theory that there must be S-oxidation activity within the basal ice. Sulphate can be also used as a terminal electron acceptor in anaerobic respiration by sulphate reducing bacteria (SRB) and sequences affiliated with *Thermodesulfovibrionaceae* were recovered in some of the basal samples (Fig 4.3). The activity of members of this sulphate reducing family can lead to the production of minerals at low temperatures where there is a high abundance of divalent cations, precipitated Ca-dolomite and Mg-calcite (Bontognali et al., 2014).

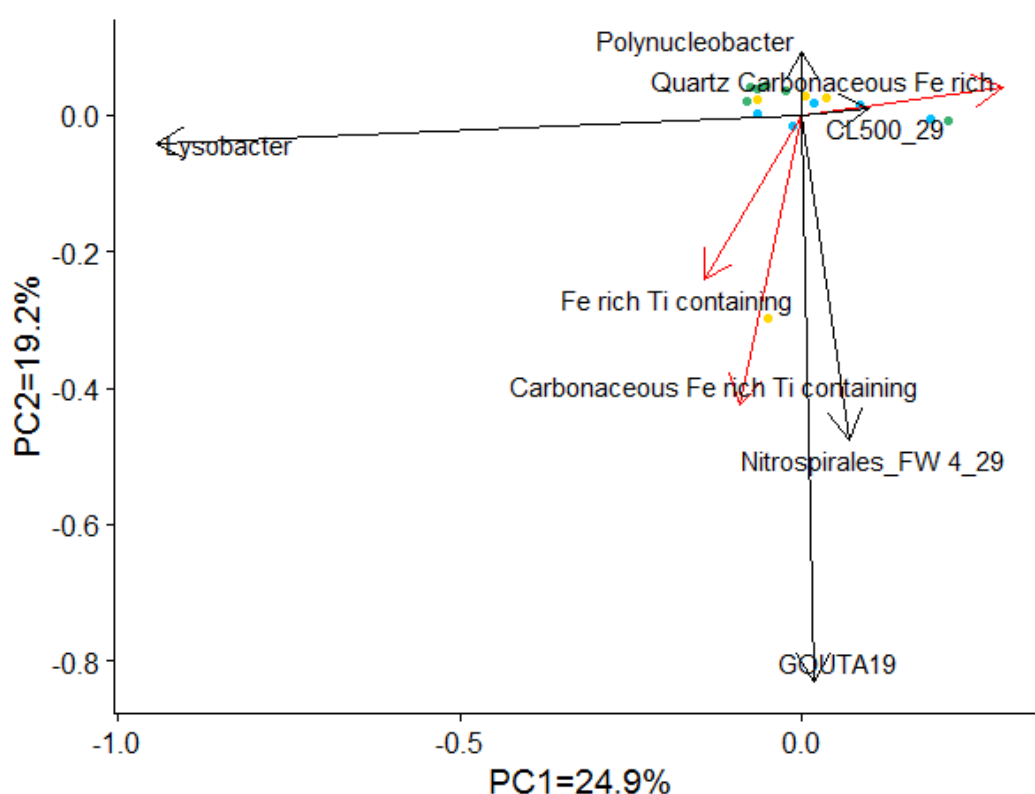
The N content in basal ice sediment was found to be very low ( $189.5 \pm 109.3$  ppm for debris bands;  $265.5 \pm 152.2$  ppm for dispersed;  $302.8 \pm 26.9$  ppm for stratified) (Fig 3.5) whilst nitrate was not detected among the minerals in SPA (Fig 3.7). This would

suggest that N appears mostly in solution as ammonium and nitrate with the identification of ammonia oxidising bacteria AOB and nitrite oxidising bacteria NOB. N was analysed in the ice matrix as  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$ . Although low,  $\text{NH}_4^+$  was found in the majority of samples. This cation can be used by chemolithotrophic species, AOB, which produce  $\text{NO}_2^-$  as a biproduct and can be subsequently used by NOB, generating  $\text{NO}_3^-$  as a final product. Interestingly nitrifying AOB (*Nitrosospira* and *Nitrosococcus*) and NOB (*Candidatus Nitrotoga*) were identified in this study among the groups that appeared with an abundance >2.5%.

The high abundance of AOB suggests that ammonia and nitrite oxidation plays a very important role in the chemolithotrophic metabolism in basal ice. Previous research in Lake Whillans and in sediment beneath the West Antarctic Ice Sheet confirmed high abundance of AOB and isotopic analysis showed evidence of this form of metabolism (Christner et al., 2014, Mikucki et al., 2016) and sequences of *cbbL* that encodes the RubisCO enzyme, affiliated with the *Nitrosospira* have also been found in subglacial sediments (Boyd et al., 2014). Very low values of  $\text{NO}_2^-$  can be explained with respect to the low stability of  $\text{NO}_2^-$ , and also due to the high abundance of NOB. Previous research has highlighted the relevance of N-oxidation as a fundamental pathway for chemoautotrophic C fixation in subglacial environments (Christner et al., 2014, Boyd et al., 2014).

Principal component analysis of bacterial OTUs across all basal ice facies samples highlighted three minerals that appear to be important in shaping the basal ice bacterial community, namely, quartz Fe-rich carbonaceous minerals, Fe-rich Ti-containing minerals, and carbonaceous Fe-rich Ti-containing minerals. Fe-rich Ti-containing minerals are not uncommon in basaltic rock (Carmichael, 1966) (Fig 7.1). Interestingly some of the bacterial OTUs, plotted as vectors seem to point in the same or opposing directions. Two groups belonging to the order *Nitrospirales* (FW4\_29 and *Thermodesulfobionaceae*-GOUTA 19) vectored to carbonaceous Fe-rich Ti-containing minerals, and Fe-rich Ti-containing minerals. Although Ti is considered to be a toxic element (Silver and Phung, 2005) and detrimental for the development of microorganisms, *Nitrospirales* have been reported to increase in the presence of Ti (McCluskey, 2016). In addition, species belonging to this order are known to interact

with Fe, including Fe-oxidising *Leptospirillum*, known to contain C-fixing chemolithotroph species (Mi et al., 2011), and alternatively Fe-reducing genera such as *Thermodesulfovibrio* (Coleman et al., 1993). If similar Fe-Ti mineral interactions are active at low temperatures in basal ice *Nitrospirales* will thrive in the presence of Ti, nullifying perceived toxicity of this element, whilst using the Fe entrapped in the same minerals for growth by either Fe oxidation by C-fixing chemolithotrophs, and/or C-consumption by Fe (III) reducers under prevailing anaerobic conditions. *Polynucleobacter* and GOUTA19, which belongs to the family *Thermodesulfovibrionaceae*, are clearly separated on PC2 and distinguished in the vector analysis, suggesting differential growth conditions.



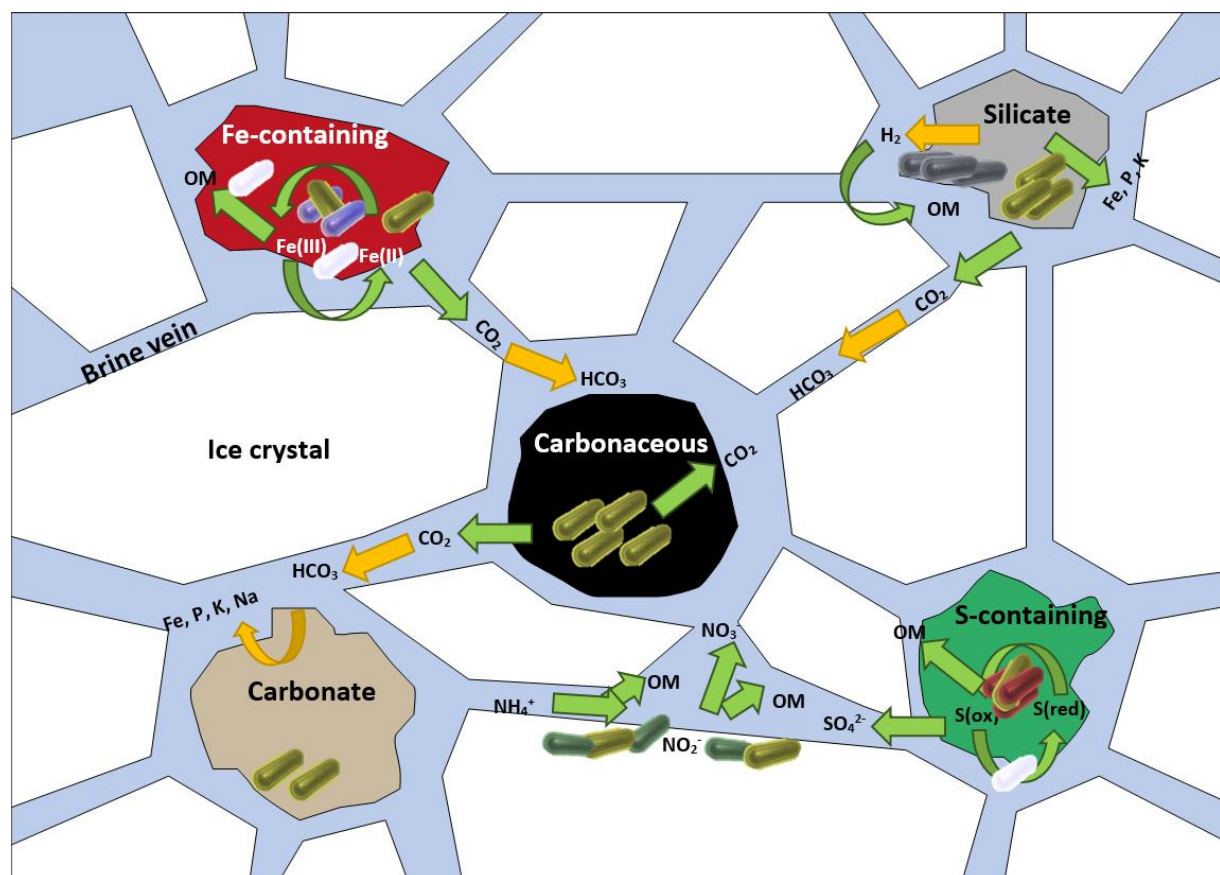
**Figure 7.1. Principal component analysis (PCA) of bacterial communities inhabiting basal ice by facies** (blue – dispersed, yellow – debris bands, green – stratified). Black vectors indicate the genus corresponding to the OTUs leading the difference between the ice facies. Mineral species analysed by SPA with significance  $p < 0.1$  are shown as bi-plotted red vectors (based on permutation tests  $n=1000$ )

Member of the genus *Polynucleobacter* have been described to be facultative anaerobes, using  $\text{NO}_3^-$  as the TEA (Hahn, Lang, Brandt, et al., 2011; Hahn, Lang, Tarao, et al., 2011; Hahn et al., 2012). *Polynucleobacter* is diametrically vectored to Ti-

containing minerals could suggest that this genus might be negatively affected by Ti. CL500-29, a bacterial taxon affiliated with *Actinobacteria*, appears to overlap with the vector corresponding to Fe-rich carbonaceous minerals. There is limited information available about this group, but sequences affiliated to CL500-29 have been identified in aquatic environments, such as deltas (Stepanauskas et al., 2003) and lakes (Wu et al., 2007). The most similar sequence (homology 99%) found in the GenBank (FJ849309.1) was identified in an Arctic stream. Although no information is available regarding the metabolism of this group, it is known that certain actinobacteria interact with Fe and are able to oxidise Fe (Hallberg et al., 2011; Jones and Johnson, 2015), some autotrophically under acidic conditions (Norris et al., 2011). Such interaction is expected in the basal ice, (see Chapter 3) due to the relative high concentration of  $\text{SO}_4^{2-}$ , indicating that the conditions would be acidic (Mader et al., 2006)

Basal ice represents a dark, oligotrophic environment (Skidmore et al., 2000), but strong evidence has been presented that prevailing chemical and geological conditions are conducive to the development of an active ecosystem. A summary of basal ice mineral bacterial interactions is presented in Figure 7.2. The high numbers of microbial cells associated to sediment tied to an abundance of bacterial sequences affiliated with chemolithotrophic species and the presence of the minerals and chemical species that are microbial products and/or substrates suggests that effectively the basal ice layer at Svínafellsjökull is an active ecosystem. In addition, it has been suggested that in subglacial environments, anoxia may occur (Skidmore et al., 2000; Hodson et al., 2008; Wadham et al., 2008), and the identification of bacterial 16S sequences affiliated to anaerobes supports this theory. The presence of sequences of microorganisms implicated in oxidation and reduction of chemical species would guarantee a recycling of elements, such as S, N, Fe, which would maintain an active ecosystem. Nevertheless, based on the abundance of Fe-containing minerals, and Fe-oxidising bacteria, it seems likely that Fe is the leading biochemical cycle in basal ice. In addition, potential Fe-oxidising bacteria were isolated using FSM media, adding strength to this theory.





**Figure 7.2. Schematic representation of physico-chemical interactions and inferred bacterial functional diversity in the basal ice ecosystem.** In red – sulphur oxidisers (*Thiobacillus*, *Gallionella*), in yellow – aerobic heterotrophs (*Lysobacter*, *Pseudomonas*), in white – anaerobic heterotrophs (*Geobacter*), green – ammonia and nitrite oxidisers (*Nitrosomona*, *Nitrosospira*) s blue – iron oxidisers (*Gallionella*, *Acidiferrobacter*), in grey – hydrogen oxidisers (*Polaromonas*, *Rhodoferrax*). Green and yellow arrows indicate respective biological and abiotic processes. Processes in which Organic Matter (OM) is produced are indicated.

Fungal cells are larger than bacterial cells, nevertheless DNA analysis from basal revealed the presence of sequences affiliated with fungi (Fig 4.5). Due to space constraints, fungi in basal should be smaller. This was reflected by the amount of sequences affiliated with yeast, or yeast-like genera, such as *Cryptococcus* (Sláviková and Vadkertiová, 2003), or *Rhodotula* (Branda et al., 2010), and *Malassezia* (Buzzini et al., 2012), and species belonging to these genera have been isolated in cryospheric environments, such as Antarctic soil or accretion ice (Buzzini et al., 2012). Filamentous fungi, such as *Alternaria*, *Cladosporium*, and *Aspergillus* were isolated from glacier ice cores from the Greenland Ice Sheet (Ma et al., 1999, Ma et al., 2000).

Fungi are known to play very important roles in geomicrobiological processes, such as rock weathering, or mineral solubilisation (Burford et al., 2003, Hoffland et al., 2004, Gadd, 2010). Members of some of the genera identified in Chapter 4 have been previously described to have weathering capabilities over rocks, such as granite, marble, basal, or sandstone, thanks to acid production (Sterflinger, 2000, Burford et al., 2003, Gadd, 2010). Some examples are *Alternaria*, which produces tenuazonic acid, *Cladosporium*, which is able to produce a variety of acids, including formic, fumaric, gluconic, and lactic acids, or *Aspergillus*, which can also produce formic, fumaric, and gluconic acids, but also acetic, itaconic, and oxalic acids (Sterflinger, 2000). The production of these acids, described as strong solubilising agents for some of the minerals identified in Chapter 3, such as feldspars, lead to the dissolution of Fe (Sterflinger, 2000, Gadd, 2010). In addition, silicates (which are the dominant minerals in the SPA analysis in Chapter 3 can be degraded by fungi, and especially by the reaction with oxalic acid, which again is produced by *Aspergillus*, *Botrytis*, and *Trichoderma*, and this genera is known to weather minerals such as olivine, and other silicates, apart from the aforementioned feldspars (Sterflinger, 2000, Burford et al., 2003).

Phosphorus, which is an element that has been described to be limiting in variety of environments, is highly insoluble, but fungi had a great potential to solubilise it due to the release of  $H^+$  to the environment, which leads to a decrease in the pH, and the chelation by certain acids, such as citric acid, like members of the genus *Aspergillus* (Sterflinger, 2000).

Fungi can also modify the redox status of metals, by oxidation and/or reduction (Gadd, 2010). Fe(II) was one of the dominant elements in the sediment entrapped in basal identified by ICP-OES in Chapter III and Fe(II) can be oxidised to Fe(III) by members of genera such as *Alternaria* or *Cladosporium*, but also some of the other members of the same genera can reduce Fe(III) back to Fe(II) (Sterflinger, 2000). Sulphides can be oxidised and the oxidised species can be accumulated by members of the genus *Aspergillus* and *Thiobacillus* (Sterflinger, 2000). The oxidation of sulphides can lead to the production of sulphuric acid, which can further weather minerals (Burford et al., 2003).

Some species of the genus *Alternaria* can reduce Fe(III) and Mn(IV), whereas some others can oxidise Fe(II). *Aspergillus* have been described to be able to oxidise sulphides (Sterflinger, 2000).

### 7.3 Relevance of basal ice derived microbial release to the glacier foreland

The deposition of microorganisms from distant sources by wind and/or precipitation has been thought to be the main driver of pedogenesis across recently deglaciated sediment (Womack et al., 2010; Chuvpichina et al., 2011; Temkiv et al., 2012). However, recent research showed that the deposition of microorganisms from glaciers plays a major role in the process of soil formation (Rime et al., 2016). From our analysis of supraglacial sediment at Svínafellsjökull,  $10^4$ - $10^5$  bacterial cell g<sup>-1</sup> of sediment were recovered and able to grow in 1:10 TSA medium, affiliated with *Stenotrophomonas rhizophila*, *Pseudomonas fluorescences* and *Cryobacterium levicorallinum* (Figs 6.6, 6.7, 6.8). The microorganisms active in supraglacial environments are likely to have been originated from sources external to the glacier and deposited by the precipitation and aeolian transport (Rime et al., 2016). Interestingly, two of the three species identified correspond to well-known plant growth promoting rhizosphere (PGPR) species, which are likely to have beneficial effects for plant colonisation and establishment in foreland moraine soils (Schmidt et al., 2012; Ye et al., 2014).

There are differing potential outcomes for microorganisms found in supraglacial environments including entrapment in subsequent snow layers in firnified-englacial ice, where adaptable communities survive and thrive whilst others remain

dormant until conditions are more habitable; (Miteva et al., 2004). Where a hydrological connection between the supraglacial and subglacial environments (e.g. moulins) exists, microorganisms can be transported through the glacier to enter the subglacial environment, in which conditions are more conducive for microbial survival (Musilova et al., 2017). During the summer melt season, microorganisms can be transported out of the glacier in water streams (run-off) (Irvine-Fynn et al., 2012). Irrespective of their fate, the presence of microorganisms in the subglacial environment provides a continual source of microbial inoculum that will eventually be deposited in the glacier foreland.

Sandy deserts are abundant in Iceland, especially the Icelandic highland, and in summertime, when the weather is drier and windy, grains can be transported and deposited on the glacier surface (Gaidos et al., 2004). Due to the close proximity of Svínafellsjökull to the southern Icelandic coast, another possible source of microbial inoculum are marine communities that can be transported and deposited as associated aerosols (Gíslason, 1990). Additionally, atmospheric deposition provides a significant inputs of C, N and other nutrients, which over time help to enrich the subglacial sediment once released (Bernasconi et al., 2011; Smittenberg et al., 2012).

Based on sediment export and microbial content of basal ice (Chapter 5, Objective A),  $10^{16}$  cells  $\text{yr}^{-1}$  were estimated to be released to the glacier margin from the basal ice. This input to the glacier margin has several crucial implications. Firstly, basal ice derived sediment has been previously suggested to represent bulk of sediment in the glacier foreland (Cook et al. 2011a) and moraines (Larson et al., 2006). The presence of an active microbiota associated with the sediment (Chapter 6, Objective B) indicates that the pedogenesis is initiated even prior to the release of sediment from the basal ice. The first crucial but well defined step involves microbial C-enrichment in the sediment, which will trigger and power growth of diverse heterotrophic bacterial and archaeal but also fungal microbial communities, many capable of mineral weathering, (Brankatschk et al., 2011) and prokaryotic N-fixation, a process requiring very high energy input (Skidmore et al., 2005). This explains the very low abundance of N-fixing bacteria identified in basal ice and recently deglaciated soil, which agrees with observations in other glaciers in Switzerland and

Peru (Nemergut et al., 2007; Duc et al., 2009; Brankatschk et al., 2011). Chemical analysis of the basal ice sediment indicated very limiting C content. However, the mineralogy and chemistry data does not preclude the likely presence of an active chemolithotrophic community which could help maintain basal ice C content and under appropriate conditions, increase the organic C content. The basal ice microbiome analysis, clearly identified sequences affiliated with chemolithotrophic bacteria and specific media for Fe-oxidisers (Linton, 2003) showed positive growth, further supporting the hypothesis that basal ice harbours an active chemolithotrophic community. It was not possible to identify cultured chemolithotrophs due to difficulties encountered relating to mixed culture and time constraints preventing further purification and 16S sequencing.

In addition to heterotrophic and chemolithotrophic bacterial and fungal communities identified via 16S and algal ITS rRNA gene sequence analyses, cyanobacterial and algae sequences were also recovered. These communities are unlikely to be active under dark conditions that prevail in basal ice (Kaštovská et al., 2007) or if so, will not be photosynthetically active due to the lack of light. However, some of the individuals belonging to these groups can be mixotrophs, exhibiting a heterotrophic metabolism when the requirement for phototrophic metabolisms are not met (Berninger et al., 1992). Whichever the case it is probable that viable algal and cyanobacterial cells entrapped in basal ice will be eventually be exported to the foreland together with the rest of the basal ice microbial community. Once freed from the ice matrix, in a light-exposed foreland, their photosynthetic capacity can start functioning, further increasing the C content in the released subglacial sediment. Initially the rates are very low and increase over time, for example, as  $0.002 \mu\text{g C g}^{-1} \text{ yr}^{-1}$  for immediately deglaciated sediment,  $0.004 \mu\text{g C g}^{-1} \text{ yr}^{-1}$  for 5 year old deglaciated soil in Midtre Lovénbreen, Svalbard (Bradley et al., 2016). Cyanobacterial cells can be easily transported and deposited by the wind (Schmidt et al., 2014). In addition, members of the *Cyanobacteria* phyla can fix N, which is a key limiting factor for soil development, and previous research showed that in very recently deglaciated soil and subglacial sediment N-fixation activity is very low, or negligible (Skidmore et al., 2000).

Overall, the most abundant phyla in basal ice were *Proteobacteria* (56.7%), *Acidobacteria* (7.8%), *Chloroflexi* (6.5%), *Actinobacteria* (5.1%) and *Bacteroidetes* (3.6%) which contrasts with the averages from general soils (Janssen, 2006), in which the most abundant phyla are *Proteobacteria* (39.2%), *Acidobacteria* (19.7%), *Actinobacteria* (12.7%), *Verrucomicrobiota* (7.0%) and *Bacteroidetes* (5.0%), and *Chloroflexi* (3.2%). The abundance of *Proteobacteria* and specially  $\beta$ -*Proteobacteria* is highly relevant in regard to soil formation. Their very versatile metabolic capabilities enable survival in adverse conditions, explaining their early high abundance in recently de-glaciated soil, later decline in abundance with soil age (Kim et al., 2017). Some of the genera found in the 16S analysis of the microbial community inhabiting basal ice showed a high diversity of  $\beta$ -*Proteobacteria*, such as *Rhodospirillum rubrum*, *Acidovorax*, *Nitrosomonas*, *Gallionella* and *Polaromonas*. The viability of these microorganisms was demonstrated by isolation of some of the members of the sub-phylum, such as *Polaromonas naphthalivorans* (which is a facultative chemolithotroph (Yagi et al., 2009)), several members of the genus *Janthinobacterium*, and members of the genus *Massilia*, *Rugamonas*, and *Acidovorax*. However, the representability of the cultivable community against the total community is low (Nichols et al., 2010; Pham and Kim, 2012; Tanaka et al., 2014), although some ecological conclusions based on the cultivable diversity can be reached. In order to increase the diversity of isolated microorganisms from basal ice, ichips were used (chapter 5). Results showed that the utilisation of ichips led to the isolation of species different to the ones isolated using exclusively traditional dilution plating methodologies, and only a minority (6) of the species isolated were found in common between both methods. Having a more diverse cultivable community means that the information obtained by cultivation is increased. *Pseudomonas* was one the genera with the highest isolated abundance among the samples, but it is not that abundant in the 16S rDNA bacteriome data. The prevalence of *Pseudomonas* in cultures in solid media has been described before, and this prevalence occurs due to *Pseudomonas* being an r-strategist, and therefore taking advantage of the resources faster than other genera present in the samples (Toscano et al., 2013). On the other hand, *Lysobacter* was the most abundant heterotrophic genus in the NGS data, but it was not observed among the isolates, although members of the genus *Lysobacter* have been isolated in diluted

TSA previously (Park et al., 2008). Nevertheless, the growth of microorganisms in general (e.g. TSA) and defined (Ferrous Sulphate Media) agar medium provides crucial information about their physiologies (Alain and Querellou, 2009). The increase in the number of microbial diversity based on NGS has displaced studies based on isolation, but there is still an intrinsic necessity to have isolates, since it is the only way in which their metabolisms can be analysed (Alain and Querellou, 2009). The fact that most of the cultured and identified bacterial isolates obtained from the Svínafellsjökull foreland are psychrotolerant, which agrees with previous literature, allows survival in a wider range of conditions, ranging from freezing temperatures inside of the ice, to mesophilic temperatures when the released sediment is exposed to solar irradiation (Wynn-Williams, 1990; Hoover and Pikuta, 2010). In addition, the majority of isolates obtained from sediment and proglacial sediment showed cold-active enzymatic activities for  $\beta$ -galactosidase, amylase and lipase. In previous research, it has been shown that recently deglaciated soils show high mineralising activities, which would agree with the expression and activity of enzyme at cold temperatures characterised in this work (Brankatschk et al., 2011). In this regard, hydrolytic activities are of great relevance within the ice matrix, allowing the recycling of nutrients (secondary production) as observed in other cryospheric environments, such as cryoconite holes (Edwards, Pachebat, et al., 2013) Analysis of the cultivable microbial community, showed that several of the isolates species have relevance for soil formation:

- a) weathering capabilities: *Pseudomonas*, *Janthinobacterium*, *Pedobacter*.  
These typically heterotrophic species can mobilise nutrients entrapped in minerals (Uroz et al., 2009; Frey et al., 2010). In addition *Polaromonas* was isolated, which is regarded as be a facultative chemolithotroph (Yagi et al., 2009).
- b) plant growth promoting rhizobacteria (PGPR): *Pseudomonas*, *Achromobacter*, *Janthinobacterium*, *Phyllobacterium* are all known to exhibit PGPR characteristics, being able to control the presence of pathogenic fungi, by the production of antifungal metabolites, or

increasing the resistance of plant to stress. (Jha and Kumar, 2009; Frey et al., 2010; Bresson et al., 2013; Sivasakthi et al., 2014)

c) N-fixation: *Achromobacter*.

#### 7.4 Glaciers as microbial conveyor belts

Due to their inherent mass flow characteristics, glaciers can be considered conveyor belts for rocks and sediments and as such the basal layer of glaciers plays a fundamental role in the process of sediment transfer (Cook et al., 2011a). Since the advent of the subglacial microbiology in the late 1990s, analogous to sediment transport, microbial transport needs to be urgently characterised.

Flowing glaciers entrain subglacial sediment from the bedrock e.g. under the icefall or when climbing against a reverse slope due to shearing forces, or associated to re-freezing (Goodsell et al., 2002; Cook et al., 2007; Cook et al., 2011b); but also from physical removal of the walls surrounding valley glaciers by scrapping, and from wind-borne sediment deposited on the glacier surface (Porazinska et al., 2004) or by avalanches (Barla and Barla, 2001), and subsequently buried by layers of snow. This sediment is then released at the glacier terminus. In addition, glaciers advance over their forelands when the climatic or dynamic conditions are right, which allows them to incorporate overridden soil into the ice matrix (Skidmore et al., 2000; Humlum et al., 2005). All these sediment sources will include associated microbial communities either attached to rock and sediment surfaces as biocrusts, cryptogamic cover and lichens (Etienne and Dupont, 2002; Bradwell, 2004; Bradwell et al., 2013). Evidence comes from previously reported mosses (Humlum et al., 2005) or algal mats (Skidmore et al., 2000). Based on historical records, Svínafellsjökull advanced across its foreland, which had been cultivated for agriculture, between the 14-19<sup>th</sup> centuries (Ives, 2007). Soil microbiota could have been entrained in the basal ice layer on numerous earlier occasions. Analysis of the 16S rRNA gene of the isolates, and BlastN comparisons revealed that a high number belong to species that have been isolated from soils, or associated with plants, which, tied to the fact that DNA corresponding to algae, cyanobacteria, and plants were identified by NGS microbiome analyses, support this theory. Apart from soil/plant microorganisms, the cultivable diversity of



Svínafellsjökull harbours microorganisms that have been isolated from rock/sediment, from water, and cryospheric environments. Further confirmation comes from the NGS analyses basal ice bacteriome identification with the most relevant OTUs from the total community: 3 out of the 5 most relevant OTUs were identified from cryospheric samples (glacier front, permafrost, and Arctic Stream), and from aquifers sediment, one of them being a basalt aquifer (Svínafellsjökull sits on top of a basaltic bedrock). This bacterial diversity would suggest that effectively Svínafellsjökull has behaved as a conveyor belt, and due to processes operating near the bedrock, has generated a mixed community from diverse environments, which could have been introduced in the glacier at different times.

### 7.5 Concluding remarks and future work

This PhD thesis has shed light about the chemical and biological nature of basal ice of the temperate Icelandic glacier, Svínafellsjökull. The low C content in basal ice sediment and the ice matrix coupled to a high abundance of bacteria and fungi suggests that the ecosystem is primarily driven by chemolithotrophy, as the NGS microbiomics analyses have confirmed, that has also been supported in the culture of psychrophilic facultative chemolithotrophs isolated at 4°C. Nevertheless, the microbial diversity assessment has been based on phylogenetic rRNA gene markers, namely 16S and ITS. This routine methodology provides a significant first insight into the basal ice system but there are obvious limitations: The approach does not discriminate between actual living cell DNA and relic DNA (Carini et al., 2016); this is important because it could potentially generate a biased view of the contemporary microbial diversity (Carini et al., 2016) inhabiting basal ice. Although no plants or plant litter were observed within the basal ice at the time of sampling, plant-derived ITS was identified in the microbiomics analysis, e.g. *Rhododendrum*, *Bryum*, and *Trifolium*, which suggests that at least part of the DNA entrapped in the basal ice can belong to this relic plant DNA. Methodologies to avoid relic DNA are available, such as RNA analysis (Blazewicz et al., 2013) or using methodologies that select only active cells DNA (Carini et al., 2016). DNA is highly stable and can persist in the environment for a long time, especially in cold temperature, but RNA is highly unstable, so if

present is more likely that it has been generated in a shorter time, and therefore could derive from active cells (Barnes and Turner, 2016).

In this thesis, chemolithotrophy has been widely discussed, and data from the microbiomics analysis indicated that this metabolism is likely to be abundant within the microbial community, especially in basal ice, but the possibilities of chemolithotrophy in sediment and moraine has also been discussed. The presence of Fe-oxidising individuals was further supported by growth in a specific Ferrous Sulphate medium (Linton, 2003) as shown in Chapter 6. However, regarding the growth times, sequencing was performed on them just after a first plating, not being able to obtain pure sequences. This made difficulted the identification of these isolates, and due to time constrains, it has been impossible to re-culture them for purity or repeat the PCRs and clone is plasmids, as pGem-T. The lack of pure culture isolation may have important implications for the understanding of the basal ice ecology, and supports observations of microbial species dependence confirmed in the co-occurrence network analysis the bacterial communities inhabiting basal ice as shown in Chapter 4. All Fe-oxidisers co-occur with other microorganisms, such as *Thiobacillus*-(*Leeia* and *Dechloromonas*) or *Gallionella*-*Acidiferrobacter*, this support the theory that also within basal ice there is a very complex community that survives forming biofilms, as widely discussed in Chapter 4 and 6. Based on the results obtained from the microbiomics analysis, probes can be developed and (Catalysed Reported Deposition) - Fluorescent In-Situ Hybridisation (CARD-FISH) methodologies can be utilised in order to demonstrate *in situ* that the groups found to co-occur actually appear together (Teske, 2005; Wilhartitz et al., 2007; Kubota, 2013). In addition, analytical techniques such as Single Particle Analysis by X-Ray Diffraction-Scanning Electron Microscope, can also inform of the chemical composition and mineralogy. In addition to that a chemolithotrophy-focus analysis can be performed, using genes implicated in CO<sub>2</sub> fixations, such as *cbbL* gene, which codes for the large subunit of the ribulose biphosphate carboxylase (Boyd et al., 2014)

Microbial-induced weathering has been one of the recurrent topics in this thesis, but only as a hypothesis based on the literature (Sharp et al., 1999; Skidmore et al., 2005; Frey et al., 2010; Yde et al., 2010; Hindshaw et al., 2016). Having a collection of

isolates from basal ice, able grow at low temperatures and affiliated with taxa that possess weathering activity, offers a unique opportunity to evaluate the potential these microorganisms have to attack minerals and mobilise metals and the implication these microorganisms could have for soil formation in glacier foreland ecosystems (Stefánsson and Gíslason, 2001; Frey et al., 2010).

Finally, the export of microorganisms from basal ice at Svínafellsjökull was estimated at  $\sim 10^{16}$  cells  $\text{ayr}^{-1}$ , but no attempt was made to quantify the alive/dead cells ratio. This could be a very intriguing possibility, analysing which fraction of the microbiota is alive and active in basal ice, and potentially being released to the glacier margin. Dead cells could be use by colonising communities once released from the basal ice matrix (Toubes-Rodrigo, Simon J. Cook, et al., 2016). Hydrolytic enzymes from heterotrophic microorganisms, such as *Lysobacter* (identified from NGS data in Chapter 4), or species belonging to some of the isolated genera in Chapter 6, such as *Streptomyces*, can then degrade these dead cells, recycling nutrients. The presence of viable cells from the basal ice layer to the margin would mean a first *in situ* inoculum, as discussed in chapter 4, 5, and 6, which would speed up the pedogenic process (Rime et al., 2016; Toubes-Rodrigo, Simon J. Cook, et al., 2016). In addition, microbiomic analysis of sediment around the glacier could be performed, addressing the difference between the microbiota in sediment entrapped in basal ice and other glacier derived niche such as supraglacial colonisation (e.g. Cryoconite hole), and also how this community evolves over time as the glacier sediment becomes incorporated into soil.

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## Appendix 1. Mineral classification code

```
.libPaths("C:/Users/55116602/Google Drive/Thesis/R functions/libraries")
```

```
library(readr)
```

```
library(plyr)
```

```
library(pca3d)
```

```
library(ggplot2)
```

```
library(cowplot)
```

```
library(reshape)
```

```
library(RColorBrewer)
```

```
library(devtools)
```

```
library(ggbiplot)
```

```
library(reshape2)
```

```
EDX      <-      read_csv("C:/Users/55116602/Google      Drive/Thesis/R  
functions/data/all_parcs.csv")
```

```
#Makes table with elements and atomic weights
```

```
el <- c("C", "O", "Na", "Mg", "Al", "Si", "P", "S", "Cl", "K", "Ca", "Ti", "Cr", "Mn", "Fe",  
"Ni", "Cu", "Zn")
```

```
aw <- c(12.012,      15.999, 22.990,      24.310, 26.980,      28.090,  
        30.970,      32.060,      35.453,      39.010, 40.080,  
        48.090,      51.996,      54.940,      55.850, 58.693,  
        63.546,      65.409)
```

```
awt <- cbind(el, aw)
```

```
awt <- as.data.frame(awt)
```

```
mol <- sweep(EDX[2:19], 2, aw, '/') #Calculates concentration in mols
```

```
mol <- cbind(ID = EDX[1], mol)
```



```
mol[is.na(mol)] <- 0
```

```
mol[20] <- rowSums(mol[2:19]) #Total mols
```

```
mol <- rename(mol, c("V20" = "total"))
```

```
mol[21] <- rowSums(mol[4:19]) #total mol - C + O
```

```
mol <- rename(mol, c("V21" = "total no C+O"))
```

```
mol[22] <- rowSums(mol[2:3])/mol[20] #% of C+ O
```

```
mol <- rename(mol, c("total.1" = "C+O"))
```

```
mol["Primary"] <- NA
```

```
mol["Sample"] <- EDX$Sample
```

```
mol["Type"] <- EDX$Type
```

```
corr <- as.vector(mol[,21]) #total molarity excluding C + O
```

```
#Sulphates
```

```
S <- mol[9]/corr
```

```
SiS <- mol[7]/mol[9]
```

```
FeS <- mol[16]/mol[9]
```

```
ClS <- mol[10]/mol[9]
```

```
SSi <- mol[9]/mol[7]
```

```
Tot <- mol[4]+mol[9]+mol[5]+mol[6]+mol[7]+mol[11]+mol[12]
```

```
SulphTable <- cbind(S, SiS, FeS, ClS, SSi, Tot)
```

```
Sulphates <- sum(SulphTable$S > 0.5 & SulphTable$Si < 0.55 & SulphTable$Fe < 0.55  
& SulphTable$Cl < 0.3, na.rm = TRUE )
```

```
Sulphates <- (SulphTable$S > 0.5 & SulphTable$Si < 0.55 & SulphTable$Fe < 0.55 &  
SulphTable$Cl < 0.3)
```

```
#Fills "Primary" table
```

```
Sulphates[SulphTable$S > 0.5 & SulphTable$Si < 0.55 & SulphTable$Fe < 0.55 &  
SulphTable$Cl < 0.3] <- "Sulphates "
```

```
#Fills "Primary" table
```

```
SulphSi <- vector()
```

```
SulphSi[SulphTable$Na > 0.7 & SulphTable[5] > 0.6 & SulphTable[5] < 2] <- "Sulphate  
Silicate "
```

```
#Halite
```

```
NaCl <- (mol[4]+mol[10])/mol[21]
```

```
SNa <- mol[9]/mol[4]
```

```
SCl <- mol[9]/mol[10]
```

```
SiCl <- mol[7]/mol[10]
```

```
HalTable <- cbind(NaCl, SNa, SCl, SiCl)
```

```
colnames(HalTable) <- c("NaCl", "SNa", "SCl", "SiCl")
```

```
Halite <- vector()
```

```
#Fills primary table
```

```
Halite[HalTable[1] > 0.5 & HalTable[2] < 0.375 & HalTable[3] < 0.5 & HalTable[4] <  
0.5] <- "Halite "
```

```
#Ti containing minerals
```

```

Ti <- mol[13]/corr
MgTi <- mol[5]/mol[13]
AlTi <- mol[6]/mol[13]
FeTi <- mol[16]/mol[13]
SiTi <- mol [7]/mol[13]
NaTi <- mol[4]/mol[13]
STi <- mol[9]/mol[13]

TiTable <- cbind(Ti, MgTi, AlTi, FeTi, SiTi, NaTi, STi)

#Fills primary table
Ti_Rich <- vector()
Ti_Rich[TiTable$Ti > 0.3 & TiTable$Mg < 1 & TiTable$Al < 1 & TiTable$Fe < 1 &
TiTable$Si < 1 & TiTable$Na < 1] <- "Ti containing "

#Si containing minerals

Si <- mol[7]/corr
MgSi <- mol[5]/mol[7]
AlSi <- mol[6]/mol[7]
FeSi <- mol[16]/mol[7]
TiSi <- mol[13]/mol[7]
NaSi <- mol[4]/mol[7]

SiTable <- cbind(Si, MgSi, AlSi, FeSi, TiSi, NaSi)

#Fills primary table

```

```

Silicate <- vector()

Silicate[SiTable$Si > 0.19 & SiTable$Mg < 1.33 & SiTable$Al < 1.33 & SiTable$Fe < 0.5
& SiTable$Ti < 0.5 & SiTable$Na < 0.7]<- "Silicate "

#Fills primary table

Quartz <- vector()

Quartz[SiTable$Si > 0.5 & SiTable$Mg < 0.2 & SiTable$Al < 0.2] <- "Quartz "


#Carbonates

Ca <- mol[12]/corr
ratio <- (mol[12]+mol[5])/corr
MgCa <- mol[5]/mol[12]
SiCa <- mol[7]/mol[12]

CaTable <- cbind(Ca, ratio, MgCa, SiCa)
colnames(CaTable) <- c("Ca", "ratio", "MgCa", "SiCa")

#Fills primary table

OtherCarbonates <- vector()

OtherCarbonates[CaTable[1] > 0.4 & CaTable$Si < 0.36] <- "Carbonates "


#Fills primary table

OtherCarbonates[CaTable$ratio > 0.5 & CaTable$MgCa > 0.33 & CaTable$MgCa < 3]
<- "Dolomite "


#Other Ca rich

SCa <- mol[9]/mol[12]

```

```
PCa <- mol[8]/mol[12]
```

```
OCRTTable <- cbind(SCa, PCa)
```

```
#Fills primary table
```

```
OtherCa <- vector()
```

```
OtherCa[OCRTTable$S > 0.25 & OCRTable$P < 0.55] <- "Ca rich "
```

```
#Iron rich
```

```
Fe <- mol[16]/corr
```

```
SiFe <- mol[7]/mol[16]
```

```
TiFe <- mol[13]/mol[16]
```

```
IRTable <- cbind(Fe, SiFe, TiFe)
```

```
#Fills primary table
```

```
FeRich <- vector()
```

```
FeRich[IRTable$Fe > 0.15 & IRTable$Si < 1.1 & IRTable$Ti < 1.43] <- "Fe rich "
```

```
#Gypsum
```

```
CaS <- (mol[12]+mol[9])/corr
```

```
ratioCaS <- mol[12]/mol[9]
```

```
NaCa <- mol[4]/mol[12]
```

```
#Fills primary table
```

```
GypTable <- cbind(CaS, ratioCaS, NaCa)
```

```
colnames(GypTable) <- c("CaS", "ratioCaS", "NaCa")
```

```
Gypsum <- vector()
```

```
Gypsum[GypTable$CaS > 0.5 & GypTable$ratioCaS > 0.25 & GypTable$ratioCaS < 0.4  
& GypTable$NaCa < 0.5] <- "Gypsum "
```

```
#Carbon rich particles
```

```
carbonaceous <- vector()
```

```
carbonaceous[mol[22] >= 0.97] <- "Carbonaceous "
```

```
#Iron titanate
```

```
AE <- rowSums(mol[4:16], na.rm=TRUE)
```

```
TiFe <- mol$Ti/mol$Fe
```

```
FeTi2 <- (mol$Fe + mol$Ti)/AE
```

```
NaTiFe <- mol$Na/(mol$Ti + mol$Fe)
```

```
MgTiFe <- mol$Mg/(mol$Ti + mol$Fe)
```

```
AlTiFe <- mol$Al/(mol$Ti + mol$Fe)
```

```
SiTiFe <- mol$Si/(mol$Ti + mol$Fe)
```

```
STiFe <- mol$S/(mol$Ti + mol$Fe)
```

```
CaTiFe <- mol$Ca/(mol$Ti + mol$Fe)
```

```
CrTiFe <- mol$Cr/(mol$Ti + mol$Fe)
```

```
MnTiFe <- mol$Mn/(mol$Ti + mol$Fe)
```

```
FeTiFe <- mol$Fe/(mol$Ti + mol$Fe)
```

```
FeTiOTable <- as.data.frame(cbind(TiFe, FeTi2, NaTiFe, MgTiFe, AlTiFe, SiTiFe, STiFe,  
CaTiFe, CrTiFe, MnTiFe, FeTiFe))
```

```
FeTiO <- vector()
```

```
FeTiO[FeTiOTable$TiFe > 0.2501 & FeTiOTable$TiFe < 0.4 & FeTiOTable$FeTi2 > 0.25  
& FeTiOTable$FeTi2 > 1.1 & FeTiOTable$NaTiFe < 0.2 & FeTiOTable$MgTiFe < 0.1 &  
FeTiOTable$AlTiFe < 0.2 & FeTiOTable$SiTiFe < 0.25 & FeTiOTable$STiFe < 0.2 &  
FeTiOTable$CaTiFe < 0.1 & FeTiOTable$CrTiFe < 0.05 & FeTiOTable$MnTiFe < 0.05]  
<- "Iron titanate "
```

```
clas <- cbind(Sulphates, SulphSi, Halite, Silicate, Quartz, FeTiO,  
Gypsum,carbonaceous, FeRich, Ti_Rich, OtherCa, OtherCarbonates)
```

```

clas[is.na(clas)] <- ""
clas[clas == "FALSE"] <- ""

mol$Primary <- apply(clas, 1, paste, collapse="")

clas2 <- clas
clas2[clas==""] <- "NA"
res_clas <- summary(clas2)
#Fill NA in Primary as "Other"
mol$Primary[mol$Primary==""] <- "Other"


#Particles per sample
sample <- levels(factor(mol$Sample))
subtotal <- vector()

for (i in 1:length(sample))
{
  tots <- table(mol$Sample == sample[i])
  subtotal[i] <- tots[2]
  print(subtotal[i])
  i = i+1
}

parts_sample <- as.data.frame(cbind(sample, subtotal))

minerals <- data.frame(table(mol$Sample, mol$Primary))
colnames(minerals) <- c("Sample", "Mineral", "Frecuency")

```

```

graphs <- data.frame()
graphs <- data.frame(unique(minerals$Mineral))
colnames(graphs)[1] <- "Mineral"
j = 1
for (j in 1:length(sample))
{
  temp <- data.frame()
  temp <- minerals[minerals$Sample == sample[j],]
  temptot <- sum(temp$Frecuency)
  temp["Percentage"] <- temp$Frecuency/temptot*100
  graphs[j+1] <- temp$Percentage
  colnames(graphs)[j+1] <- sample[j]
  j = j+1
}

melted <- melt(graphs, id.vars =c("Mineral"))
write.csv(x=melted, "melted_part.csv")

colnames(melted) <- c("Cluster", "Sample", "Frequency")

min_ana <- melted
for (i in 1:nrow(min_ana))
{

  if(grepl("S", min_ana$Sample[i])){
    min_ana$type[i] <- "Stratified"}
  if(grepl("D", min_ana$Sample[i])){
    min_ana$type[i] <- "Dispersed"}
  if(grepl("B", min_ana$Sample[i])){
    min_ana$type[i] <- "Band"}
}

```



```

print(i)
}

mean_mins <- dcast(min_ana, Cluster ~ type, value.var="Percentage", fun.aggregate
= mean)

sd_mins <- dcast(min_ana, Cluster ~ type, value.var="Percentage", fun.aggregate =
sd)


means_sd_mins <- cbind(mean_mins, sd_mins[2:4])
write.csv(means_sd_mins, "means_sd_minerals.csv")

MEcolnames(melted) <- c("Cluster", "Sample", "Percentage")

#melted$Cluster      <-      factor(melted$Cluster,      levels      =
melted$Cluster[order(melted$Percentage, decreasing = F)]) #Reorder by frequency


#graphs colours
Cacol <- c("gold3", "gold")
carbcol <- rev(brewer.pal(9, "Greys"))
dolcol <- "orange4"
otherCa <- "lemonchiffon1"
ironcol <- rev(brewer.pal(6, "Reds"))
gypcol <- "aquamarine"
halicol <- rev(brewer.pal(3, "Purples"))
othercol <- "floralwhite"
silcol <- colorRampPalette(brewer.pal(19, "Blues"))
sulpcol <- colorRampPalette(brewer.pal(11, "Greens"))
Ticol <- c("grey", "gray47", "gray80")


cols <- c(Cacol, carbcol, otherCa, dolcol, ironcol, gypcol, halicol, othercol,
rev(silcol(17)), rev(sulpcol(10)), Ticol, "white")


#bar chart

```

```

p <- ggplot(melted, aes(x = Sample, y = Frequency, fill = Cluster)) +
  geom_bar(stat = "identity", width = 0.5)+
  theme(legend.position = "bottom", legend.text = element_text(size=16), axis.text.x
= element_text(hjust = 1,angle = 60, size = 16))+
  scale_fill_manual(values = cols)+
  labs(fill="")

```

p

```

#Plot pca
mins_pca <- dcast(min_ana, Cluster ~ Sample, value.var = "Percentage")
pca_data <- t(mins_pca)
sample_names <- pca_data[1,]
colnames(pca_data) <- sample_names
pca_data<- as.data.frame(pca_data[-1,])
pca_data <- apply(pca_data, c(1,2),as.numeric)
pc <- prcomp(pca_data)

```

```

samples_ids<-as.data.frame(rownames(pca_data))
type <- vector()
for (i in 1:nrow(samples_names))
{

```

```

  if(grepl("S", samples_ids[i,])){
    type[i] <- "Stratified"}
  if(grepl("D", samples_ids[i,])){
    type[i] <- "Dispersed"}
  if(grepl("B", samples_ids[i,])){

```

```

    type[i] <- "Band"}
  print(i)
}

```

```

pca2d(pc, biplot = T, group = type, show.labels = samples_ids$`rownames(pca_data)` ,
show.ellipses = T)

```

```

#calculating difference between ice samples

```

```

mins <- melt(graphs, id.vars = "Mineral")

```

```

mins[4] <- NA

```

```

colnames(mins) <-c("mineral", "samples", "percentage", "type")

```

```

for (i in 1:nrow(mins))

```

```

{

```

```

  if(grepl("S", mins$samples[i])){

```

```

    mins$type[i] <- "Stratified"}

```

```

  if(grepl("D", mins$samples[i])){

```

```

    mins$type[i] <- "Dispersed"}

```

```

  if(grepl("B", mins$samples[i])){

```

```

    mins$type[i] <- "Band"}

```

```

  print(i)

```

```

}

```

```

cast_mins <- dcast(data = mins, mineral ~ type)

```

```
fit <- aov(data=mins, percentage ~ type)
```

```
pca <- prcomp(tcasted[-1])
```

```
type <- c("band", "band", "band", "band", "dispersed", "dispersed", "dispersed",  
"dispersed", "dispersed", "stratfied", "stratfied", "stratfied", "stratfied")
```

```
g <- ggbiplot(pca_sample, groups = type, ellipse = T, var.axes = T, scale = 0, var.scale  
= T, varname.adjust = 2, varname.abbrev = T)
```

```
g
```

```
#export
```

```
write.csv(x = mol, file = "IdParticles.csv")
```

```
write.csv(x=graphs, file="Mineral_abundance2.csv")
```

```
write.csv(x = res, file = "PrimaryClust.csv")
```

```
save_plot(filename = "PrimaryClust.wmf", p)
```